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High-rate anaerobic treatment of dilute wastewater at psychrophilic temperature

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High-rate anaerobic treatment of dilute wastewater at psychrophilic temperature

by

Gouranga Chandra Banik

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Civil Engineering (Environmental Engineering)

Major Professor: T. Al Austin

Iowa State University

Ames, Iowa

1998

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LIST OF ABBREVIATIONS

The following abbreviations are used in this dissertation:

θ	= Temperature correction coefficient (1.02-1.09)
θ	= Hydraulic retention time, day
ρ_p	= Density of particle, Kg/m ³
ρ_w	= Density of water, Kg/m ³
μ	= Specific growth rate of biomass, 1/time
μ_m	= Maximum specific growth rate of biomass, 1/time
ε	= Dynamic viscosity of water, Kg/m/s
ASBR	= Anaerobic sequencing batch reactor
BA	= Biocarbonate alkalinity, mg/L as CaCO ₃
BOD ₅	= Five-day biochemical oxygen demand, mg/L
COD	= Chemical oxygen demand, mg/L
d_p	= Diameter of particle, mm
dX/dt	= Biomass growth rate, mg/L/time
dS/dt	= Substrate utilization rate, mg/L/time
E	= Activation energy, cal/mole/°K
F/M	= Food to microorganism ratio, 1/day
g	= Acceleration due to gravity, m/s ²
[H ₂ CO ₃ *]	= Carbonic acid concentration, Mole/L
[HCO ₃ ⁻]	= Biocarbonate concentration, Mole/L
[H ⁺]	= Hydrogen ion concentration, Mole/L
K	= Substrate utilization rate, 1/day
K _d	= Biomass endogenous decay rate, 1/time
K _s	= Half-velocity constant, mg/L

K_o	= Frequency factor of reaction. constant
K_1, K_2	= Reaction rate constants at temperatures T_1 and T_2 in $^{\circ}\text{C}$
K_H	= Henry's law constant. $\text{kPa}\cdot\text{m}^3/\text{g}$
MLSS	= Mixed liquor suspended solids. mg/L
MLVSS	= Mixed liquor volatile suspended solids. mg/L
NFDM	= Non-fat-dry milk
OLR	= Organic loading rate. g/L/day
P_{H_2}	= Hydrogen partial pressure at equilibrium state. kPa
Q	= Influent wastewater flowrate. m^3/day
R	= Universal gas constant. $8.314 \text{ J/mole/}^{\circ}\text{K}$
SEM	= Scanning electron microscopy
SRT	= Solids retention time. day
T	= Temperature. $^{\circ}\text{K}$ or $^{\circ}\text{C}$
TA	= Total alkalinity. mg/L as CaCO_3
TSS	= Total suspended solids. mg/L
V	= Volume of reactor. L
VFA	= Volatile fatty acids. mg/L
V_s	= Particle settling velocity. m/s
X	= Biomass concentration. mg/L
Y	= Biomass yield coefficient. $\text{mg of biomass/mg of utilized substrate}$

ABSTRACT

The application of anaerobic technology has been mostly directed towards the treatment of medium and high strength wastewater at mesophilic and thermophilic temperatures. The possibilities of anaerobic treatment could be increased if the process could be applied at low temperatures for treatment of various types of dilute wastewater. Problems limiting the treatment of dilute wastewater at psychrophilic temperature are related to the wastewater and the reactor design.

This research evaluated the application of the anaerobic sequencing batch reactor (ASBR) at various low temperatures and hydraulic retention times (HRTs). Anaerobic treatment of dilute wastewater was studied using three, laboratory-scale ASBRs, each with an active volume of six (6) liters. The reactors were fed a synthetic substrate made from non-fat dry milk supplemented with nutrients and trace metals. The COD and BOD₅ of the feed was 600 mg/L and 285 mg/L, respectively. Pseudo steady-state performance data were collected at reactor temperatures of 25, 20, 17.5, 15, 12.5, 10, 7.5 and 5 °C over a period of two years. Hydraulic retention times (HRTs) were maintained at 24, 16, 12, 8 and 6 hours. Results showed that the ASBR process was capable of achieving in excess of 90% soluble COD and BOD₅ removal at temperatures of 25 °C and 20 °C at all HRTs. At the low temperature of 5 °C and the six hour HRT, soluble COD and BOD₅ removals were 62% and 75%, respectively. At the intermediate temperatures from 20 °C down to 5 °C and HRTs between 24 and 6 hours, removal of soluble organics ranged between 62 and 90 % for COD and 75 and 90 % for BOD₅. In all cases, solid retention times (SRTs) were high enough to maintain good performance.

In addition, the methanogenic activity, microstructure and size distribution of the ASBR granules were also evaluated. Results revealed no significant variation in microbial structure for the different temperature conditions. Granules at 15 and 25 °C exhibited uniform structure, predominantly *Methanothrix*-like microorganisms, while granules at 5 °C indicated

the existence of a layered structure. This reinforces the hypothesis that layering is largely substrate specific, but also suggests that temperature may have a role. The mean size of the granules increased as temperatures decreased from 25 to 15 °C then decreased with a further reduction in temperature. The equivalent diameter of granules was 2.0-3.3 mm for all temperatures and HRTs.

This research also evaluated the effects of low temperatures on the microbial kinetic parameters K , K_s , K_d , Y_g and μ_m . The values of K , K_d and μ_m were found to decrease, and the half saturation constant, K_s was found to increase with the decrease of temperature in accordance with the Arrhenius equation. Temperature coefficient, θ values for K , μ_m , K_d and K_s were 1.083, 1.062, 1.076 and 1.05 respectively indicating a linear interrelationship among them. Through the determination of kinetic parameters, a design engineer may be able to predict the performance of the treatment systems of ASBR over a wide range of psychrophilic temperatures.

CHAPTER 1

GENERAL INTRODUCTION

A. Introduction

Anaerobic treatment of waste and wastewater is presently accepted as a proven technology, and its application is growing each year [Nyns, 1994; Wheatley, 1990]. Several kinds of wastewaters believed to be unsuitable for anaerobic treatment are now treated with advanced reactor systems [Franklin et al., 1992; Franklin et al., 1994a, 1994b, Nagano et al., 1992; Narayanan et al., 1993a, 1993b]. In addition, recent research shows that various recalcitrant compounds, like chlorinated aliphates, chlorinated aromates, nitroaromates and other xenobiotics, can be degraded under either anaerobic or in aerobic-anaerobic sequences (Pavlostathis, 1994; Field et al., 1994). These recent developments show that the full potential of anaerobic digestion is still underestimated.

One of the major contributions to the success of anaerobic wastewater treatment is the introduction of high-rate reactors in which biomass retention and liquid retention are uncoupled, such as the anaerobic sequencing batch reactor (ASBR) [Dague and Pidaparti, 1991], the upflow anaerobic sludge blanket (UASB) reactor [Lettinga et al., 1980], the anaerobic filter (AF) [Young and McCarty, 1969; Young and Yang, 1989], the fluidized bed reactor [Jeris, 1983], the anaerobic baffled reactor (ABR) [Bachmann et al., 1985], the anaerobic attached film expended bed (AAFEB) reactor [Switzenbaum and Jewell, 1980] and the anaerobic gas lift reactor (AGLR) [Beefink and Staugaard, 1986]. High-rate reactors can accommodate very high organic loading rates because of the high concentration of bacterial mass and the sufficient biomass-substrate contact. Among the above reactor systems, the ASBR is undoubtedly becoming successful for its simplicity, high treatment efficiency and economy [Dague and Pidaparti, 1991; Sung and Dague,

1995; Wirtz and Dague, 1995]. In most cases, the anaerobic reactor is only a pre-treatment unit of a complete wastewater treatment system.

The possibilities of anaerobic treatment technology could be increased if the process could also be applied at different range of temperature including psychrophilic conditions. Psychrophilic treatment could be an attractive alternative, particularly when the treatment is necessary for tropical and sub-tropical regions. In the psychrophilic range of temperatures ($< 25^{\circ}\text{C}$), reaction rates are slower than thermophilic or mesophilic ranges of temperature, leading lower substrate utilization and lower biomass growth rate. However, the biomass decay rate is also lower in the psychrophilic range, and this balances the lower biomass growth rate. Solubility of gas and viscosity of liquid are also important factors for treatment at low temperatures.

The anaerobic processes can also be applied for treatment of low strength or dilute wastewater. Low strength or dilute wastewaters are those wastewaters which exhibit less than 1000 mg COD/L, may contain a variety of biodegradable compounds such as simple short-chain volatile fatty acids (VFA), alcohols and carbohydrates, as well as proteins, fats and long-chain fatty acids (LCFA) in some cases (Kato et al., 1994) . One important example of low strength soluble wastewaters are effluents from alcoholic and soft drinking bottling industries, paper recycling and paper making mills, fruit and vegetable canneries, malting and brewing processes [Carveiro et al., 1986; Isaac and McFiggans, 1981; Lettinga and Hulshoff, 1986]. Some soluble wastewaters may have a wide concentration range since the COD of industrial effluents depends largely on the technological processes and reuse or recycling of water.

The problems with the treatment of dilute wastewaters are as low substrate concentrations occurring inside the granules, possible presence of dissolved oxygen, and low temperatures. The reactor design requires high retention of highly active biomass and good wastewater-biomass contact. Both requirements are dependent on mixing intensity and retention time. Low substrate levels inside the bioreactor and the granules result in

low activity of the biomass, which can be explained by Monod kinetics [Monod, 1949]. According to Monod kinetics, the specific growth rate (μ) and specific methanogenic activity depend on the substrate concentration. Since it is desired to have the highest possible COD elimination in wastewater treatment, so the reactor effluents should have a low substrate concentration. Therefore, an optimized treatment performance means that reactor biomass should have high specific activity and low K_S values. In the case of granules, there are substrate transport limitations. The lower substrate gradient between inside the biomass and the bulk liquid phase, will result in inefficient utilization of the biomass.

Diffusion is one of the factors that has a considerable effect on treatment of dilute wastewaters at different temperatures. The values of the diffusion coefficient, expressed by Fick's law, decrease with temperature and size of solute, and are also dependent on the solute concentration [Geankoplis, 1983; Welty et al., 1969]. Low natural mixing and low methane production are also a problem for treatment of dilute wastewaters at low temperatures. All the problems related to the treatment of dilute wastewaters at low temperature are discussed in greater detail in the literature review.

The Anaerobic Sequencing Batch Reactor (ASBR) which has been developed at Iowa State University, was used for this research. Initial experiments with the ASBRs were conducted in 1989 and 1990 by Habben[1991], and Dague and Pidaparti [1991]. Since that time, the ASBR has been applied at laboratory scale to several different wastewaters, including starch wastewaters, furfuraldehyde wastewaters, landfill leachate and swine wastes. Additionally, fundamental research has been conducted on a number of operational aspects of the ASBR. Past research has been aimed at defining appropriate height to width ratios, mixing requirement, biosorption, granulation and operating temperatures. The ASBR has also been demonstrated at pilot-scale for a starch wastewater. Two full-scale ASBRs for treating swine waste and slaughterhouse wastes

are in the construction phase. Further details concerning ASBR research is found in the literature review.

It is hypothesized that the ASBR process would be an efficient treatment system for the treatment of low strength wastewater at psychrophilic temperatures without the addition of any chemicals or heat. To confirm the hypothesis, it was decided to conduct a detailed investigation on ASBR treatment of low-strength wastewaters at various temperatures (25, 20, 17.5, 15, 12.5, 10, 7.5 and 5 °C) and at hydraulic retention times (24, 16, 12, 8, 6 and 4 hr HRT).

B. Objectives

The main objective of this study was to evaluate the performance of the ASBRs treating dilute wastewater (COD strength 600 mg/L) at psychrophilic temperatures of 25 to 5 °C and at HRTs of 24, 16, 12, 8, 6 and 4 hrs.

The following specific studies were conducted for the achievement of the overall objectives:

A. Elucidate the effect of low-temperature on ASBR treatment of dilute wastewater.

- Examine the effect of temperature in terms of TCOD, SCOD and BOD₅ removals
- Examine the effect of temperature on biomass inventory
- Examine the effect of temperature on volatile fatty acids concentrations and methane production.

B. Elucidate the effect of hydraulic retention time (HRT) on ASBR treatment of dilute wastewater.

- Evaluate the effect of HRT on TCOD, SCOD and BOD₅ removals at various low temperatures
- Evaluate the effects of HRT on biomass inventory

- Evaluate the effect of HRT on volatile fatty acids concentrations in the reactors and methane production at various temperatures.
- C. Elucidate the effect of temperatures and HRTs on the structure, composition and methanogenic activities of granules.
- D. Evaluate the effect of temperature and HRTs on the microbial kinetic parameters assuming ASBR dilute wastewater treatment at psychrophilic temperatures follow the Monod kinetics. Determinations of the values of the kinetic parameters at each condition is very important in successful design and operation of biological systems.
- E. Determine the effect of temperatures and HRTs on the granule size diameter and density. The granule size diameter considers here as a characteristics parameter. The ASBR granule size distribution will be used to develop a model.

C. Dissertation Organization

The outline of this dissertation is as follows:

Chapter 2 includes the literature review. The effects of psychrophilic temperatures and low substrate concentrations on treatment performances is described. Some microbiology and biochemistry of granules is also discussed. The principle and characteristics of ASBR treatment is explained. This chapter also elaborates on the performance of various biological reactors in treating low-strength wastewater at low temperatures.

Chapter 3 describes the laboratory set-up of the study in greater detail. It also includes a description of the parameters measured during this research and the methods used. The procedure and frequency of measurement of the parameters will also be mentioned in this chapter. The characteristics of the substrate (non-fat-dry-milk) and Ames tap water will be included as well.

Chapter 4 includes the performance of ASBRs treating non-fat -dry-milk (600 mg COD/L) at different psychrophilic temperatures and at different HRTs in respect of organic removals, methane production, solid levels and VFA concentrations. The chapter also explains the feasibility of the ASBR treatment in such conditions.

Chapter 5 includes the structure and methanogenic activities of granules at different temperatures and HRTs conditions. Layer formation and composition of bacteria is explained in this chapter.

Chapter 6 describes the variation of granule size distribution and density at various conditions. In this chapter, the granule size will be characteristic parameter of granule size distribution. The granule size at various conditions may be used here to develop a model.

Chapter 7 describes the microbial kinetic parameters and their variation at different temperature and HRT conditions. The determined parameters is compared with other studies in this chapter as well.

The dissertation is concluded by a general **Summary and Discussion** of the obtained results in the different chapters.

Chapters 4, 5, 6 and 7 are presented here according to the Journal formats of Water Environment Research, Water Science & Technology, American Filtration & Separation and Environmental Technology respectively. Chapters 1, 2 and 3 are presented according to the ISU dissertation format.

CHAPTER 2

LITERATURE REVIEW

This literature review consists of several sections. These sections include: A) History, microbiology and physiology of anaerobic treatment, B) Environmental and operating parameters, C) Anaerobic Sequencing Batch Reactor (ASBR) operating principles and mechanisms, D) High-rate anaerobic treatment systems, and E) Performance of various biological systems of treating dilute wastewater under low temperature conditions.

A. Anaerobic Treatment Of Wastewater

1. History of Anaerobic Treatment

Anaerobic organisms were first discovered in 1861 by Louis Pasteur during his research on fermentation processes [Dague, 1961; Prescott et al., 1990]. The utilization of anaerobic microorganisms in waste treatment started in 1881 with the development of Mouras' Automatic Scavenger [Jewell, 1987; McCarty, 1981].

In its early application, anaerobic treatment was applied on sludge digestion. Experience with anaerobic digestion of sludges where 50 % reduction of solids was possible even at long stabilization times. It led researchers to lose interest in the application of anaerobic treatment of liquid wastes. A report by Batch [1931] states that anaerobic waste treatment was only applicable for sludge stabilization and not for the treatment of liquid wastes.

However, a report by Fullen [1953] showed successful anaerobic treatment of packing house waste in a system called the 'anaerobic contact process'. McCarty [1964a] mentioned that the belief that anaerobic treatment was an inefficient process was a fallacy related to the experience with sludge digestion where most of the organic material being treated were not susceptible to biological degradation, and only about 50 percent reduction in solids is possible. McCarty [1964a, 1964b, 1964c, 1964d, 1968, 1981] differentiated anaerobic

treatment from anaerobic digestion, and became pioneer in the anaerobic treatment. McCarty [1968] referred anaerobic digestion as solid destruction, and anaerobic waste treatment as general waste treatment or organic removal.

Researchers recognized in the early 1950's that the maintenance of high population of biomass in the reactor would be necessary for successful anaerobic treatment of liquid wastes. One of the earliest researches on the retention of biomass in anaerobic reactor was reported by Stander [1950]. He found that the separation of bacteria from the effluent stream and returning them to the reactor was helpful in maintaining a large population of methanogens in his laboratory treatment of various wastewaters from the fermentation industry [Stander and Snyder, 1950]. The principle of effluent sludge separation was later demonstrated by Stander in a full-scale treatment of winery wastewater in an anaerobic 'clarigester' reactor which employed a settling tank for the return of effluent solids to the reactor [Iza et al., 1991]. The mechanism of effluent solids capturing and recycling to the reactor was used in the development of the first high-rate anaerobic reactor 'the anaerobic contact process' by Fullen [1953].

2. Anaerobic and Aerobic Treatment

Aerobic treatment has been widely used for low-strength domestic wastewater treatment and also for many industrial wastes. Aerobic bacteria, because of the high energy yield characteristics, grow very rapidly as compared to the anaerobic bacteria. Aerobic bacteria also has small amount of energy from degradation of a given waste material. Aerobic bacteria convert some (approximately 1/3 to 1/2) of the waste material to carbon-di-oxide and water, with the remaining energy to cell synthesis. The result of this process is the production of large quantities of biomass which must be further stabilized before stabilization through the anaerobic digestion. In addition, aerobic organisms require molecular oxygen to grow and reproduce. The addition of oxygen to the aerobic wastewater treatment system, such as an activated sludge system, results in significant operating costs.

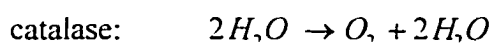
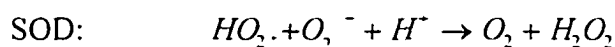
On the other hand, anaerobic microorganisms require no oxygen, and are, in fact, inhibited or killed by free oxygen. Therefore, there is no requirement for oxygen in anaerobic waste treatment. Since anaerobic microbes reproduce at relatively low rates, as most of the energy is lost for the production of methane, the biomass reproduction rate is low which further results in lower biomass disposal costs. Another advantage is that nutrient requirements are lower for anaerobic bacteria, owing to their lower reproduction rates. An additional benefit to anaerobic treatment is the production of methane, which contains (about 65-85%) of the energy of the original waste compound. That is, a significant portion of the energy of a waste can be captured in the form of methane, which can then be used to produce electricity or burned to release heat [Dague, 1981; McCarty, 1964].

There are, however, a few disadvantages to anaerobic treatment when compared to aerobic treatment. The first is the slow growth rates of the methanogenic bacteria, resulting in long start-up periods of anaerobic systems. The phenomenon has traditionally been confused with low substrate removal rates. The underlying principle behind the slow methanogenic growth rates is that methanogens derive very little energy through methanogenesis, and, therefore, require long time to reproduce. That notwithstanding, the long start-up period for anaerobic systems is a significant problem for the widespread use. Many industries simply are not willing to wait for several months or even years for the waste treatment to reach the design loads. The second disadvantage is the requirement for higher operating temperature for efficient anaerobic waste stabilization for lower methanogenic activity at low temperature. In general, industry doesn't like to spend money for heating the anaerobic system.

The problem of low temperature, therefore, was selected as one of the items to be addressed in this research. That is, systems of anaerobic treatment to achieve higher organic removal of a given wastewater at psychrophilic temperature were to be examined in this research.

3. Anaerobic Bacteria

a. Oxygen tolerance. Anaerobic biological treatment involves the use of a microbial consortium to reduce the organic content of a waste stream in the absence of molecular oxygen. The toxicity of oxygen is not due to molecular oxygen, O_2 , but rather to the reduced forms of oxygen, including hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and hydroxyl radical ($\cdot OH$) [Holland et al., 1987]. Superoxide dismutase (SOD) converts the superoxide anion to oxygen and hydrogen peroxide, and catalase converts hydrogen peroxide to oxygen and water in most aerobic organisms by the following equations:



The built-in-defense mechanism of SOD, catalase and quenching molecules are sufficient to protect the cells from reduced form of oxygen in normally growing aerobic organisms [Holland et al., 1987; Zubay, G., 1983]. Strict anaerobic bacteria are always inhibited or killed by the presence of free oxygen. There is, however, a wide range of oxygen resistance in anaerobic bacteria. Additionally, it has been shown that many anaerobes such as facultative bacteria contain defense systems similar to aerobic bacteria, including catalase and SOD. The degree of toxicity of oxygen to anaerobes depend on several factors such as rate and mode of oxygen reduction, protective enzymes, cell composition, repair mechanisms etc. [Holland et al., 1987]. A gradual increase of oxygen supply did not significantly interfere on the performance of anaerobes. Very interesting was that at low oxygen supply, the methane production as well as organic removal is stimulated by 20% [Gerritise et al., 1990].

b. Microbial pathways. The end product in the anaerobic methanogenic conversion of organic pollutants is methane and carbon dioxide. According to the McCarty [1964], Henze and Harremoes [1983] and Thiele [1991], three major biological reaction steps are

involved in anaerobic treatment. The microbial population in an anaerobic system, in the absence of sulfate and nitrate, are therefore, made up of three trophic groups in terms of reaction occurring. These groups include hydrolytic fermentative organisms, syntrophic acetogenic organisms and methanogenic fermentation organisms. The hydrolytic bacteria break down complex organic matter into simple, low molecular weight end products such as lactate, ethanol, acetate, formate, hydrogen, propionate and butyrate in the hydrolytic step. In the acetogenic reaction step, the acetogens oxidize hydrolytic fermentation end products like ethanol, propionate, butyrate and benzoate into acetate, formate and hydrogen. Finally, in the methanogenic reaction step, the acetate, formate and hydrogen are converted into methane gas and carbon dioxide.

Different researchers explain the metabolic pathways involving different kind of anaerobic bacteria in different ways, although the end product is methane. Schematic is shown in Figure 2-1 showing the conversion of organic matter to methane and carbon dioxide, based on the three trophic microbial groups presented by Thiele [1991]. McCarty [1964a] shows the pathway of chemical oxygen demand (COD) transformation in the conversion of organic matter to methane in percent. This is illustrated in Figure 2-2. Zeikus [1979, 1981] divided the microbial population in anaerobic reactor into four trophic groups: hydrolytic bacteria, H_2 -producing acetogenic bacteria, homoacetogenic bacteria and methanogenic bacteria. The schematic modified by Zeikus is shown in Figure 2-3. Holland et al. [1987] shows a pattern of carbon flow in anaerobic treatment which is shown in Figure 2-4. These figures are simple sketch, but can be complicated by the cross feeding of vitamins and amino acids. But all of the above Figures 2-1 through 2-4, explain the relationships and mechanisms that go in the anaerobic treatment.

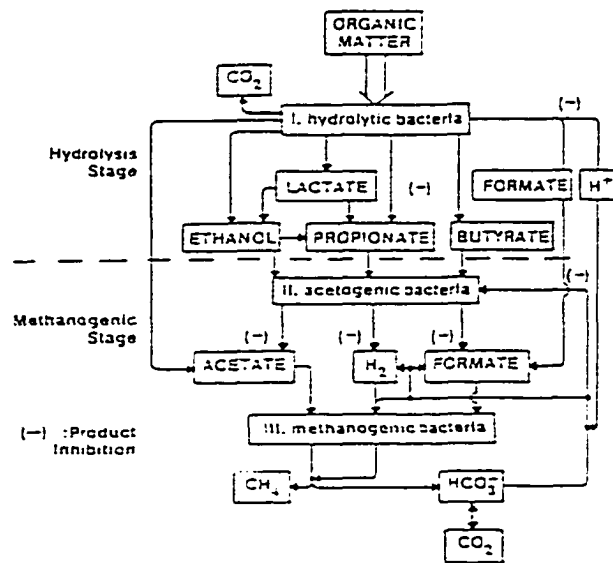


Fig. 2-1. Conversion of organics to methane based on three trophic group [Theile, 1991]

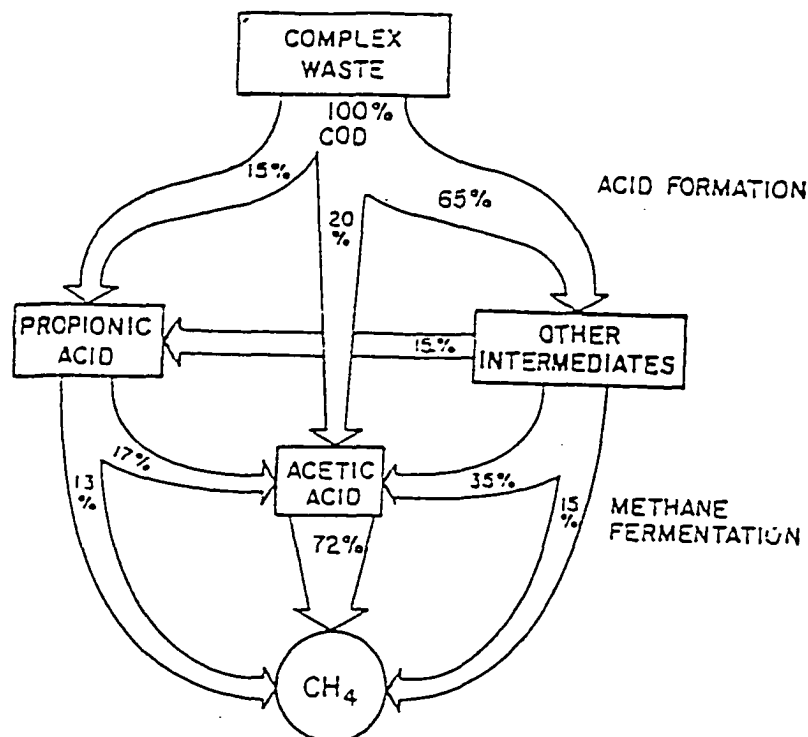


Fig. 2-2. Methane fermentation of a complex organic waste [McCarty, 1964a]

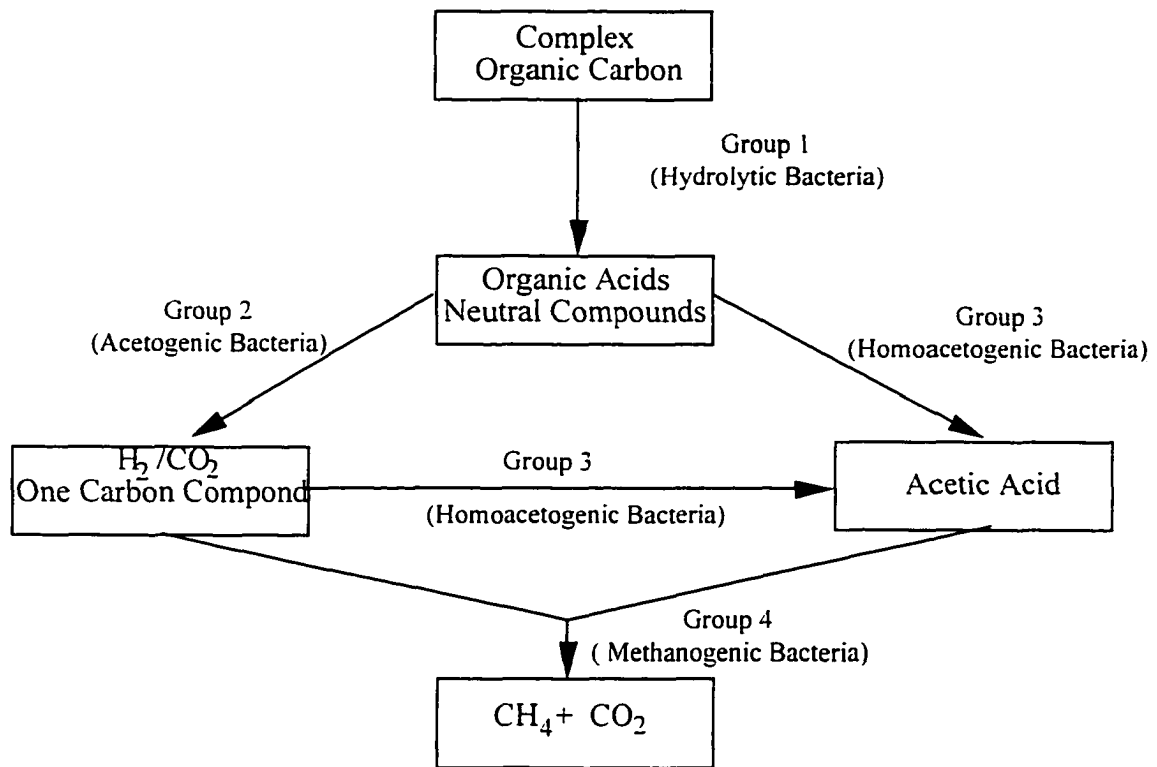


Fig. 2-3. Schematic of four levels of microbial species [Zeikus 1979, 1981]

Novaes [1986] classified the metabolic processes and microbial groups involved in anaerobic treatment into five groups which are shown in Figure 2-5. The five groups include: fermentative bacteria, H_2 -producing bacteria acetogenic bacteria, H_2 -consuming acetogenic or homoacetogenic bacteria, CO_2 -reducing methanogenic bacteria, and acetoclastic methanogenic bacteria. The schematic of Zeikus [1979] and Novaes [1986] describe modern understanding of the microbial groups in the anaerobic treatment. They show that propionate can not be used by methanogens, as previously believed. secondly, they simplified the syntrophic relationship between the acetogens and methanogen. Thirdly, they simplified the two-stage process (acetogens and methanogen) involved in anaerobic treatment and the importance of interspecies hydrogen transfer in anaerobic process control.

c. Physiology. The physiological characteristics of bacteria representative of four trophic groups in anaerobic treatment are shown in Table 2-1 by Zeikus [1979]. The table also explains that how much time is required to become double in number, including fermentative products. For instance, hydrolytic bacteria with nutrients heterotrope+ and catabolic substrate cellulose require 7 days to become double to produce ethanol, acetate and lactic. Table 2-1 shows the typical population densities for the different group of bacteria with genetic identity, most commonly found in anaerobic treatment.

d. Hydrolytic fermentative bacteria. In most of the anaerobic treatment or ecosystem, the incoming substrate is fairly complex, consisting of polymer, such as polysaccharides, proteins, lipids, nucleic acids, or a combination of these. The hydrolytic

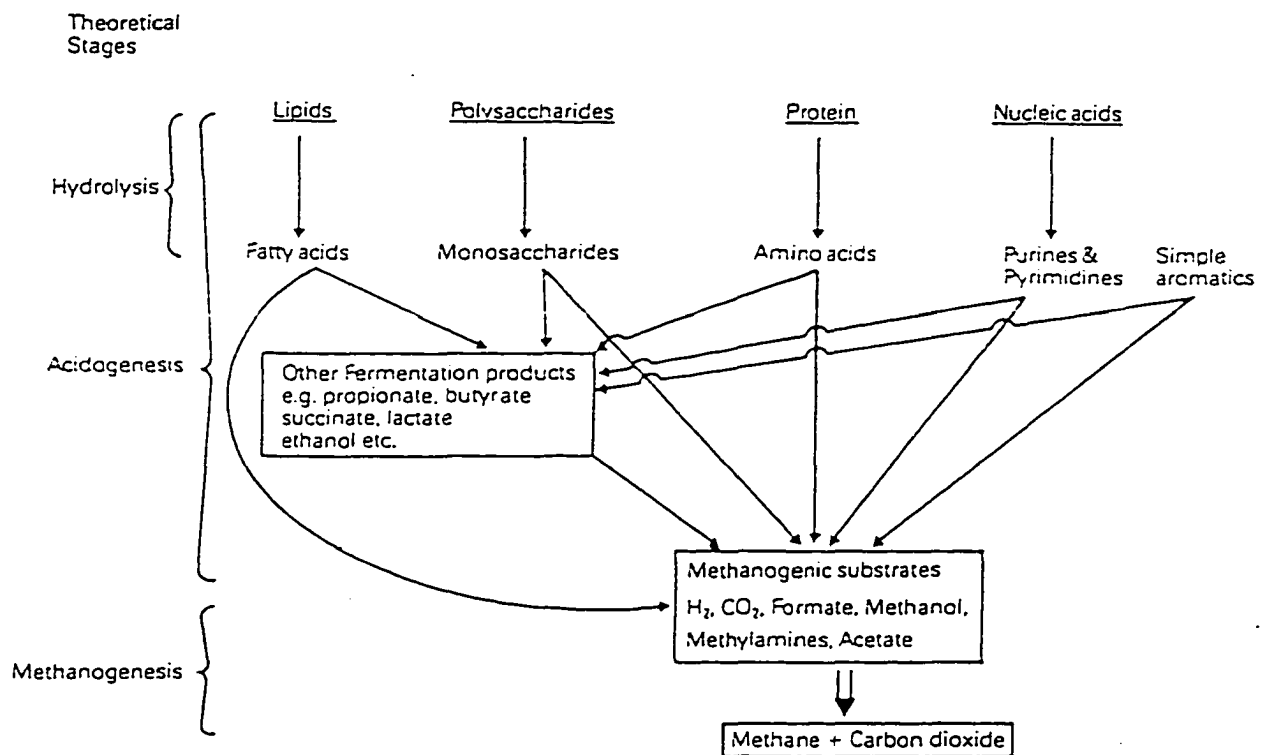


Fig. 2-4. Pattern of carbon flow in anaerobic digestion [Holland et al., 1987]

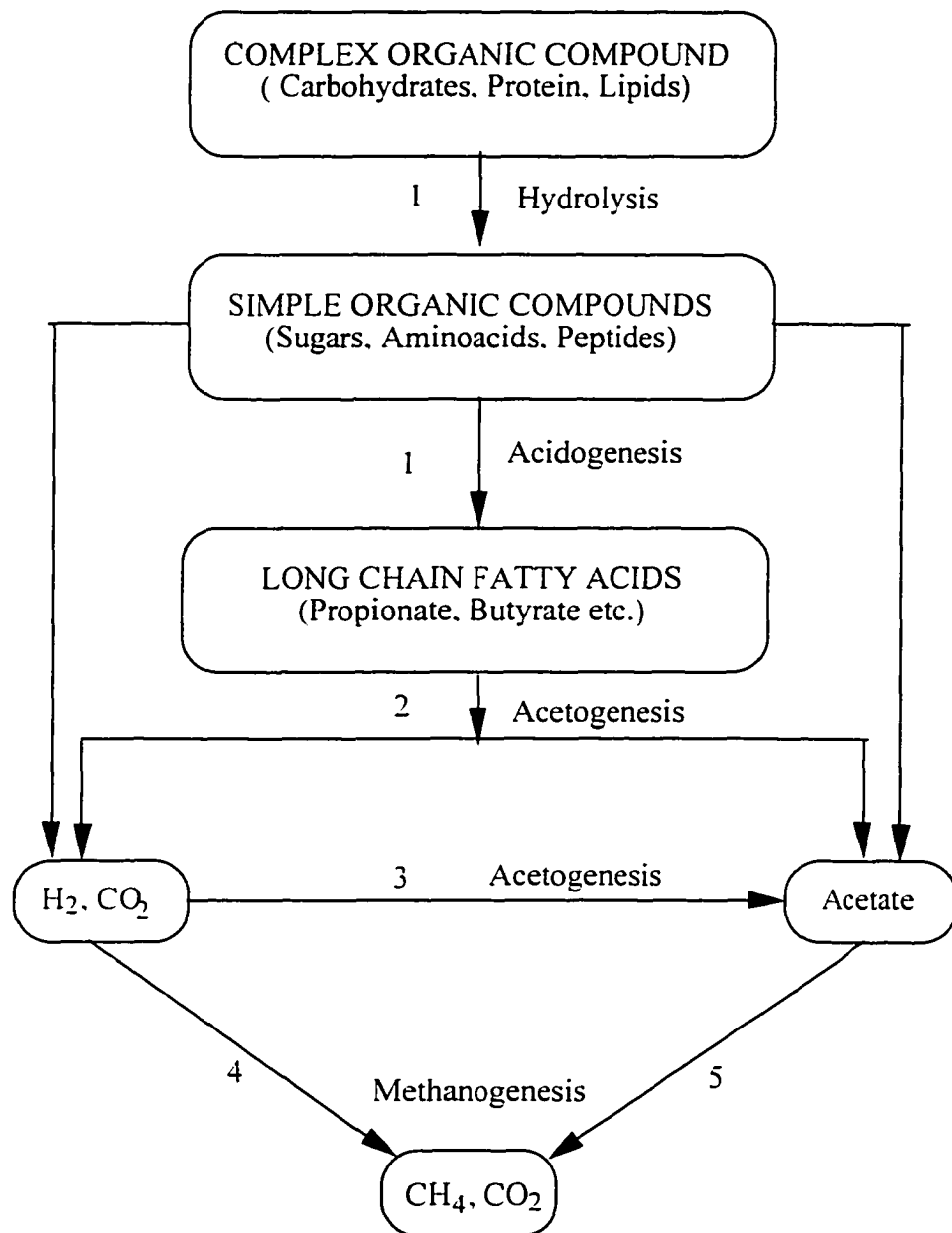


Fig. 2-5. Metabolic and microbial groups involved in anaerobic treatment [Novaes, 1986]. [1) Hydrolytic bacteria; 2) H₂-producing bacteria; 3) H₂-consuming bacteria; 4) CO₂-reducing bacteria; 5) Acetoclastic methanogenic bacteria.]

bacteria convert those high molecular organics to monomers and oligomers of the respective starting compounds. The produced monomers and polymers is then transported to the interior of the cells and fermented into a variety of products such as ethanol, acetate, propionate, carbon dioxide, hydrogen, NH_4^+ , and S^{2-} [Bryant, 1979; Chyi et al., 1992; Daniels, 1984; Novaes, 1986]. These bacteria are, therefore, referred to as acidogenic bacteria or acidogens. This include obligate anaerobes such as *Clostridium Bacteroides*, and *Ruminococcus species* and facultative anaerobes such as *E. coli* and *Bacillus species* [Daniels, 1984].

Table 2-1. Physiological characteristics of of four major trophic groups bacteria in anaerobic treatment [Zeikus, 1979].

Organism	Tropic Group	Nutrition^a	Catabolic Substrates^b	Doubling Time^c	Fermentation Products^d
<i>Clostridium thermocellum</i>	Hydrolytic bacteria	Heterotroph+	Cellulose Cellobiose	7 2	H_2/CO_2 , ethanol Acetic, lactic
'S organism'	H_2 -producing acetogenic	Heterotroph+	Pyruvate Ethanol	- -	H_2/CO_2 , ethanol Acetic, H_2
<i>Acetobacterium woodlii</i>	Homoacetogenic	Mixotroph+ H_2/CO_2	Fructose, lactic	6 24	Acetic Acetic
<i>Methanobacterium thermoautotrophicum</i>	Methanogenic bacteria	Autotroph-	H_2/CO_2 CO	2-4 30	CH_4 , CO_2
<i>Methanosarcina barkeri</i>	Methanogenic bacteria	Mixotroph-	H_2/CO_2 CH_3NH_2 CH_3COOH	10-12 >24	CH_4 , CO_2

^a (+) Growth factors required; (-) no growth factors required

^b Substrates shown are not the exclusive source metabolized by the individual species

^c Apparent doubling time under optimal growth conditions

^d Major end products

Table 2-2. Typical population densities for the five groups of anaerobic bacteria
[Zeikus, 1979]

Group	Genetic Identity	Number (per ml)
Hydrolytic Bacteria	Majority unidentified	
Total	Gram-negative rods	10^8 - 10^9
Proteolytic	Eubacterium	10^7
Cellulolytic	Clostridium	10^5
Hydrogen-producing acetogenic bacteria	unidentified Gram- negative rods	10^6
Homoacetic bacteria	Clostridium Acetobacterium	10^5 - 10^6
Methanogens	Methanobacterium	10^6 - 10^8
	Methanospirillum	
	Methanococcus	
	Methanosarcina	
	Methanothrix	
Sulfate reducers	Desulfovibrio	10^4
	Desulfotomaculum	

The hydrogen produced by the acidogens (Figure 2-6) is removed by the other groups of bacteria in a well-functioning anaerobic system. The products from the work of the acidogenic bacteria will be in a relatively oxidized state such as acetate and carbon dioxide. But if the system will not remove the hydrogen produced by the acidogens, more reduced products such as propionate, butyrate lactate will be formed [Bryant, 1979]. Lipids and proteins are also hydrolyzed by the acidogens to their respective end products [Bryant, 1979; Zehnder, 1988]. Lipids are generally split into free fatty acids, glycerol and other non-fatty-acids products. The non-fatty-acids are fermented to additional acids, carbon dioxide and H_2 . Unsaturated fatty acids are hydrogenated to form saturated fatty acids. Generally, proteolytic bacteria hydrolyze peptide bonds in protein, resulting in the production of peptides and free

amino acids, and results in the production of short-chain and branched-chain fatty acids, NH_3 after fermentation.

Factors important for proper function of the acidogenic bacteria are substrate solubility and complexity, pH and temperature [Bryant, 1979; Chyi et al., 1992; Zehnder,

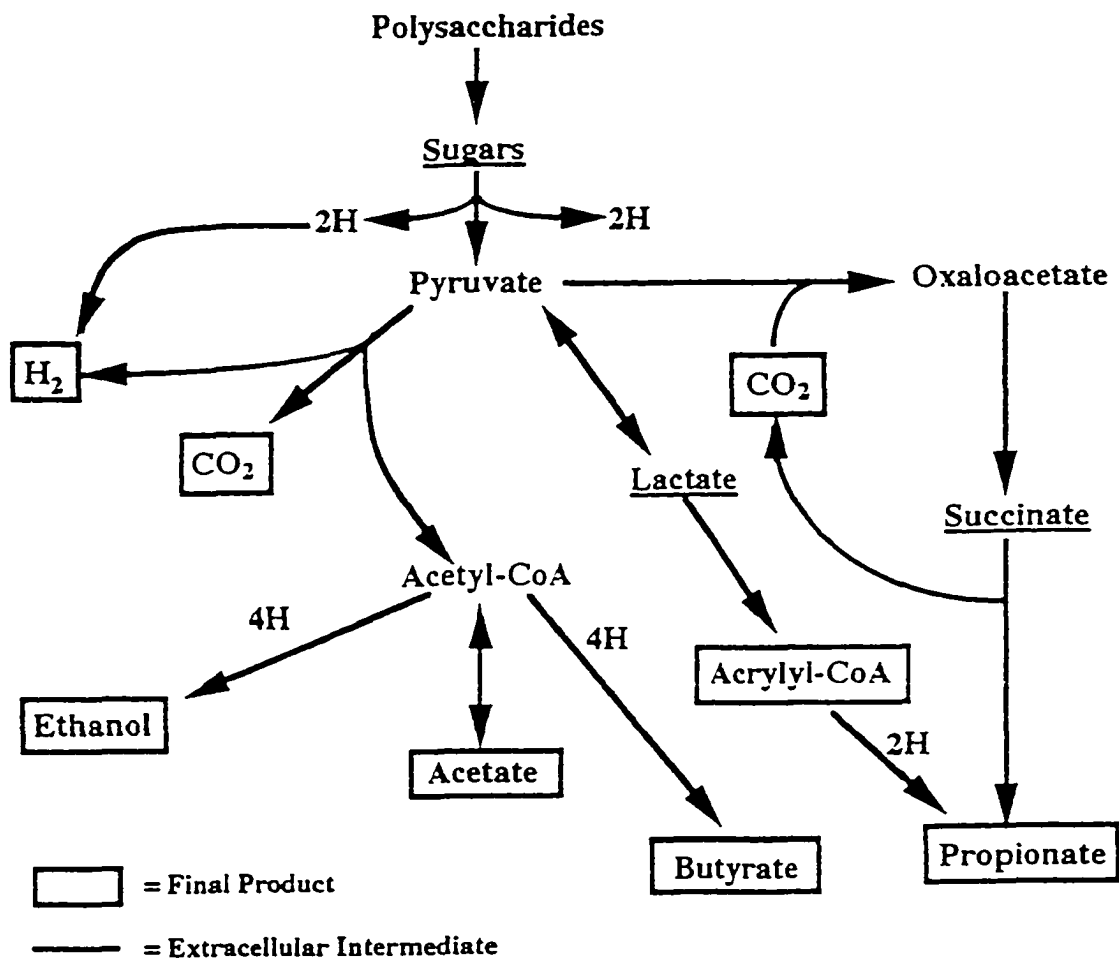


Fig. 2-6. Simplified pathways for polysaccharide fermentation by the acidogenic bacteria [Bryant, 1979].

1988]. The pH of the system has a significant effect on the rate of hydrolysis and solubilization of large polymers. The optimum pH for the hydrolytic bacteria is in the range of 5.6 to 8 [Bryant, 1979; Zehnder, 1988]. Chyi et al. [1992] determined the optimal pH as 5.2 to 6.0 with anaerobic solubilization experiments. The solubility of the substrate is also important as more soluble molecules generally undergo hydrolysis more readily than insoluble molecules. The effect of temperature is not well documented as since most studies on hydrolysis have been conducted with rumen bacteria at ambient temperatures.

e. Acetogenic syntrophic bacteria. The acetogenic bacteria oxidizes hydrolytic fermentation products to acetate and other fatty acids [Bryant, 1979; McCarty, 1964; Zehnder, 1988]. The acetogens is the important intermediate group in the conversion of organics to methane because 70% of substrate is going to methane (Figure 2-2). In general terms, there are two basic groups of anaerobic acetogenic bacteria- the proton-reducing acetogens which use hydrogen as their electron sink to form molecular hydrogen called acetogenic dehydrogenation, and the hydrogen-utilizing acetogens which use hydrogen in the reduction of more oxidized molecules to form acetate called acetogenic hydrogenation. Both the group of acetogens can exist in syntrophic association. It is essential that methanogens should maintain an extremely low pressure of hydrogen in order to grow for the hydrogen producing bacteria. catabolize hydrolytic end products and produce hydrogen. One explanation for the dependency of the hydrogen producing bacteria on the methanogens have been presented by Bryant, [1979], Chyi et al. [1992], and Zehnder, [1988]. Table 2-3 summarize the change in the free energy of the reactions for cometabolism of hydrogen producing acetogens in pure culture with hydrogen utilizing methanogens.

It can be seen from the Table 2-3 that the cometabolism of propionate to acetate, carbon dioxide and H_2 (reaction A) and the catabolism of butyrate to acetate and H_2 (reaction B) would not proceed alone because of the highly positive free energy for both reactions [Bryant, 1979]. However, the H_2 -utilizing reactions by methanogens (reaction C) would

proceed because of its highly negative free energy. Therefore, when the H₂-producing bacteria is placed in syntrophic association with the H₂-utilizing bacteria (sum A+C, and sum of B+C), then the combined reactions (reaction D and E) become energetically favorable enough to occur [Bryant, 1979]. The reactions to favor H₂-producing bacteria that is with a negative free-energy, the H₂ partial pressure must be less than 10⁻³ atm for the use of the butyrate and 10⁻⁴ atm for the use of propionate. That is, propionate acetogens is more sensitive to H₂ partial pressure than butyrate acetogens. However, McCarty [1981] believes that the utilization of propionate by H₂-producing bacteria requires H₂ partial pressure not exceeding 10⁻⁶ atm.

Table 2-3. Stoichiometry and change in free-energy of the reaction for catabolism of propionate and butyrate by H₂-producing acetogens impure culture with H₂-utilizing methanogens [Bryant, 1979].

A-Propionate -catabolising acetogenic bacterium		
$\text{CH}_3\text{CH}_2\text{COO}^- + 3 \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{H}^+ + 3 \text{H}_2$		$\rightarrow +18.2 \text{ Kcal/reaction}$
B-Butyrate-catabolizing acetogenic bacterium		
$\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + \text{H}^+ + 2\text{H}_2$		$\rightarrow 11.5 \text{ Kcal/reaction}$
C-H ₂ -Utilizing methanogenic bacterium		
$\text{HCO}_3^- + 4\text{H}_2\text{O} + \text{H}_2 \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$		$\rightarrow 32.4 \text{ Kcal/reaction}$
D-Syntrophic association of A and C		
$4\text{CH}_3\text{CH}_2\text{COO}^- + 3\text{H}_2\text{O} \rightarrow 4\text{CH}_3\text{COO}^- + \text{HCO}_3^- + \text{H}^+ + 3\text{CH}_4$		$\rightarrow -24.4 \text{ Kcal/reaction}$
E-Syntrophic association of B and C		
$2\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + \text{HCO}_3^- + \text{H}_2\text{O} \rightarrow 4\text{CH}_3\text{COO}^- + \text{CH}_4 + \text{H}^+$		$\rightarrow -9.4 \text{ Kcal/reaction}$

f. Methanogenic bacteria. The methanogens utilize the end products of the other groups of bacteria, mainly acetate, carbon dioxide, and H_2 to form methane and CO_2 . These are generally considered the final group of bacteria in the anaerobic degradation of organic substrates [Holland et al., 1987]. The methanogens are the main utilizer of acetate (70%) of the carbon in an anaerobic system. The energy contained on COD basis in the methane produced by the methanogens normally represents 90% of the initial energy of the original substrate, and therefore, represents the majority of stabilization of a given organic waste material. Organic matter could not be anaerobically stabilized without the methanogens. More over, methanogens neutralize the acetic condition (low pH) of the system by stabilizing the organics and producing methane. Taxonomically, the methanogens have been grouped into five orders and nine families [Blaut, 1994]. A list of some of the known methanogenic bacteria is shown in Table 2-4.

The methanogens mentioned in the Table 2-4, have different optimal temperature for their growth and survival. The details of optimal temperature of different groups of bacteria was mentioned [Schmit, 1997]. The temperature ranges in which methanogens can live range from 4 °C to 97 °C, which means, anywhere methanogens can be found. Daniels [1984] states that methanogens live in waterlogged soils, the gut of animals, sewage sludge, manure piles, marine and fresh water sediments, and hot springs.

The substrate utilized by the methanogenic bacteria consist of approximately 3 group of substrate as methyl, acetoclastic and CO_2 type substrates. Methyl group substrates include: methanol, methylamine, dimethylamine, trimethylamine, methylmercaptan, dimethylsulfide; acetoclastic substrate as acetate; and CO_2 type substrates as CO , CO_2 and formate. In addition to a carbonaceous substrate, methanogens need sulfur, cobalt, iron, molybdenum, nickel, magnesium, potassium, selenium, and sodium chloride in sufficient amounts for growth [Zehnder, 1988]. The methanogens generally live within a pH range of 6 to 8, but some can survived at a pH upto 9.0 [Zehnder, 1988; Protorious, 1983].

Table 2-4. The Methanogens [Brummeler et al., 1985]

Germ	Morphology	Number of Species	Substrate for Microorganisms
GROUP I			
<i>Methanobacterium</i>	long rods	8	H ₂ + CO ₂ , formate
<i>Methanobrevibacter</i>	short rods	3	H ₂ + CO ₂ , formate
GROUP II			
<i>Methanothermus</i>	rods	2	H ₂ + CO ₂ , reduces S ⁰
GROUP III			
<i>Methanococcus</i>	irregular cocci	5	H ₂ + CO ₂ , formate
GROUP IV			
<i>Methanomicrobium</i>	short rods	2	H ₂ + CO ₂ , formate
<i>Methanogenium</i>	irregular cocci	3	H ₂ + CO ₂ , formate
<i>Methanospirillum</i>	spirilla	1	H ₂ + CO ₂ , formate
GROUP V			
<i>Methanoplanus</i>	plate-shaped	2	H ₂ + CO ₂ , formate
<i>Methanospaera</i>	cocci	1	CH ₃ OH + H ₂
GROUP VI			
<i>Methansarcina</i>	irregular cocci	3	H ₂ + CO ₂ , formate, CH ₃ OH, methylamines
<i>Methanolobus</i>	irregular cocci	3	CH ₃ OH, methylamines
<i>Methanoculleus</i>	irregular cocci	4	H ₂ + CO ₂ , formate, alcohols
<i>Methanotherix</i>	rods/filaments	3	acetate
<i>Methanosaeta</i>	rods/filaments	1	acetate
GROUP VII			
<i>Methanopyrus</i>	rods in chain	1	H ₂ + CO ₂
GROUP VIII			
<i>Methanocorpusculum</i>	irregular cocci	3	H ₂ + CO ₂ , formate, alcohols

The most biochemical reactions involved in methane formation are shown in Table 2-5. From the Table 2-5, it can be seen that methanogens have a relatively low energy yield from -32.5 KJ/mole to -185.5 KJ/mole, depending on the substrate. This low energy can sometimes be a problem for the methanogens depending on the substrate, for competing with sulfate reducing bacteria and denitrifying bacteria. For instance, SRB can outcompete methanogens for substrate which contain larger amount of sulfate, for their high affinity of hydrogen.

Table 2-5. Substrates and energy yielding reactions for methanogenic bacteria
[Zehnder et al., 1988; Blaut et al. 1994]

Reaction	ΔG° (KJ/mol CH ₄)
$4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	-130.4
$4\text{HCOO}^- + 2\text{H}^+ \rightarrow \text{CH}_4 + \text{CO}_2 + 2\text{HCO}_3^-$	-126.8
$4\text{CO} + 2\text{H}_2\text{O} \rightarrow \text{CH}_4 + 3\text{CO}_2$	-185.1
$4\text{CH}_3\text{OH} \rightarrow 3\text{CH}_4 + \text{CO}_2 + 2\text{H}_2\text{O}$	-102.5
$4\text{CH}_3\text{NH}_3^+ + 2\text{H}_2\text{O} \rightarrow 3\text{CH}_4 + \text{CO}_2 + 4\text{NH}_4^+$	-74.0
$\text{CH}_3\text{COO}^- + \text{H}^+ \rightarrow \text{CH}_4 + \text{CO}_2$	-32.5
$(\text{CH}_3)_2\text{S} + \text{H}_2\text{O} \rightarrow 1.5\text{CH}_4 + 0.5\text{CO}_2 + \text{H}_2\text{S}$	-74.0
$(\text{CH}_3)_2\text{NH}_2 + 2\text{H}_2\text{O} \rightarrow \text{CH}_4 + \text{CO}_2 + \text{NH}_4^+$	-74.0

The methanogens always maintain low hydrogen partial pressure (i.e., less than 10^{-5} atm) in the anaerobic system, which promotes growth of other type of bacteria. At the low hydrogen partial pressure, the thermodynamics of the acedogenic and acetogenic bacteria is favorable for their growth, and the pH of the anaerobic environment can be maintained within the optimal range (6.5-7.5) for their growth [Zehnder et al. 1988].

The important function of methanogens are to stabilization of acetate, CO₂ by regulating the balance of : 1) proton ; 2) electron and 3) nutrient [Zeikus et al 1981]. The significance of these regulation can be seen in the Table 2-6. The methanogens by regulating proton, electron and nutrient, create a favorable conditions for efficient organic stabilization.

Table 2-6. Role of methanogens in anaerobic system [Zeikus et al. 1979]

Function performed	Metabolic reaction	Process significance
Proton regulation	$\text{CH}_3\text{COO}^- + \text{H}^+ \rightarrow \text{CH}_4 + \text{CO}_2$	<ul style="list-style-type: none"> •Maintain pH •Remove toxicmetabolite
Electron regulation	$4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	<ul style="list-style-type: none"> •Increase metabolite rates •Prevent accumulation of toxic metabolites
Nutrient regulation	Excretion of growth factor	<ul style="list-style-type: none"> •Stimulates growth of hetrotrophes

g. Methanogenesis. Different pathways are involved in methanogenesis depending on substrate type and formation. As discussed earlier, most of the organic waste contain carbohydrates (starch), lipids (fat), and protein. The steps of degradation and final conversion to methane are presented in Figure 2-7 through 2-9. The steps are hydrolytic, acetogenic and methanogenic which were discussed earlier. As shown in Figures 2-7 through 2-9, the degradation pathways of these three organic components reveal that the hydrolytic step requires more reactions for the breakdown of proteins to organic acids in comparison to carbohydrates and lipids [Chanin et al., 1961].

Substrate types as carbon dioxide, methyl and acetate (previously mentioned) has also certain differences and similarities in different segments of pathways. But for the limitation of the scope of this research, only one pathways involving methyl substrate is illustrated here (Figure 2-10). Methyl substrates donate their methyl groups to a vitamin B12 protein to form

methyl-B12, which then transfers the methyl group to CoM. Reducing equivalents for the reduction of methyl-CoM are derived from the oxidation of other methanol molecules to CO_2 [Brook et al., 1991; Zehnder, 1988].

h. Interspecies interaction. The successful operation of an anaerobic process requires the presence of the right population of all required microbial species. In actual environments, however, all groups of bacteria work together to fully stabilize organic material within their environment. In fact, various groups depend on the other groups to provide utilizable substrates, to degrade metabolic end-products, and to maintain appropriate conditions (i.e., pH) in the environment.

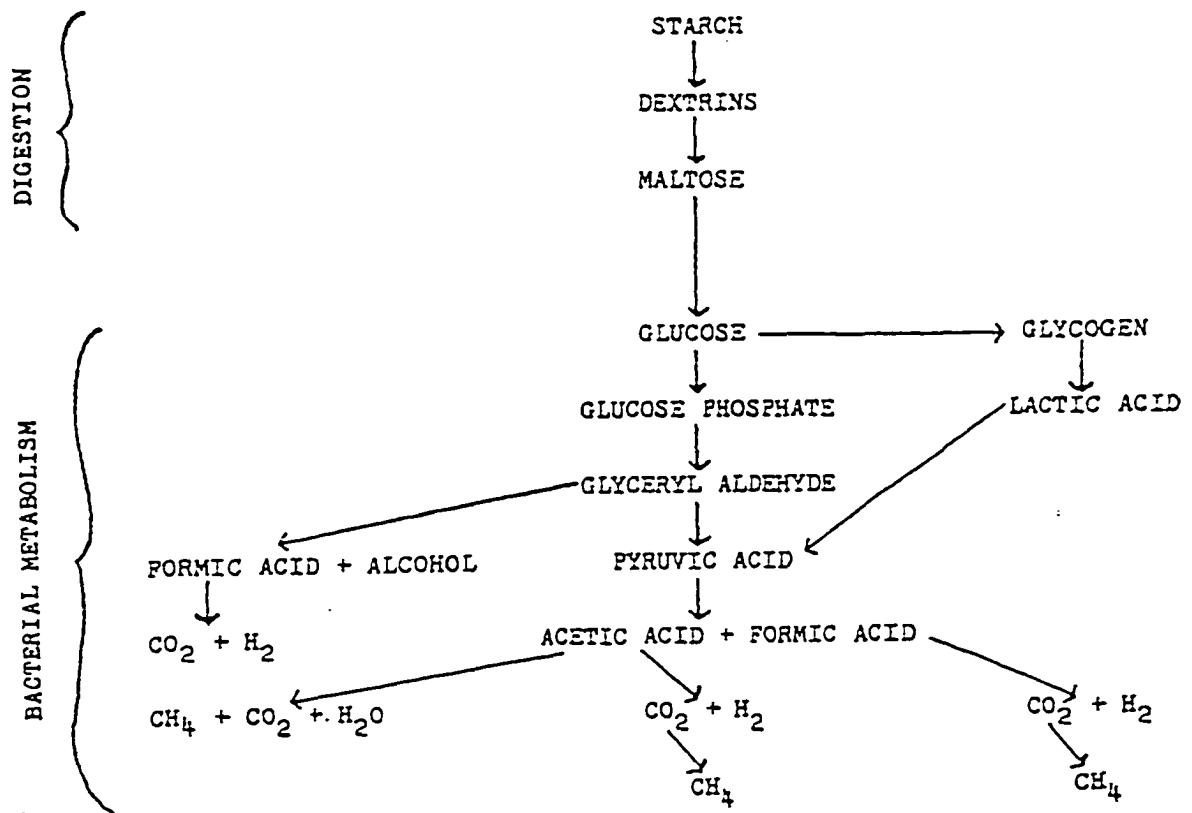


Fig. 2-7. A possible pathway for anaerobic degradation of starch [Chanin, 1961]

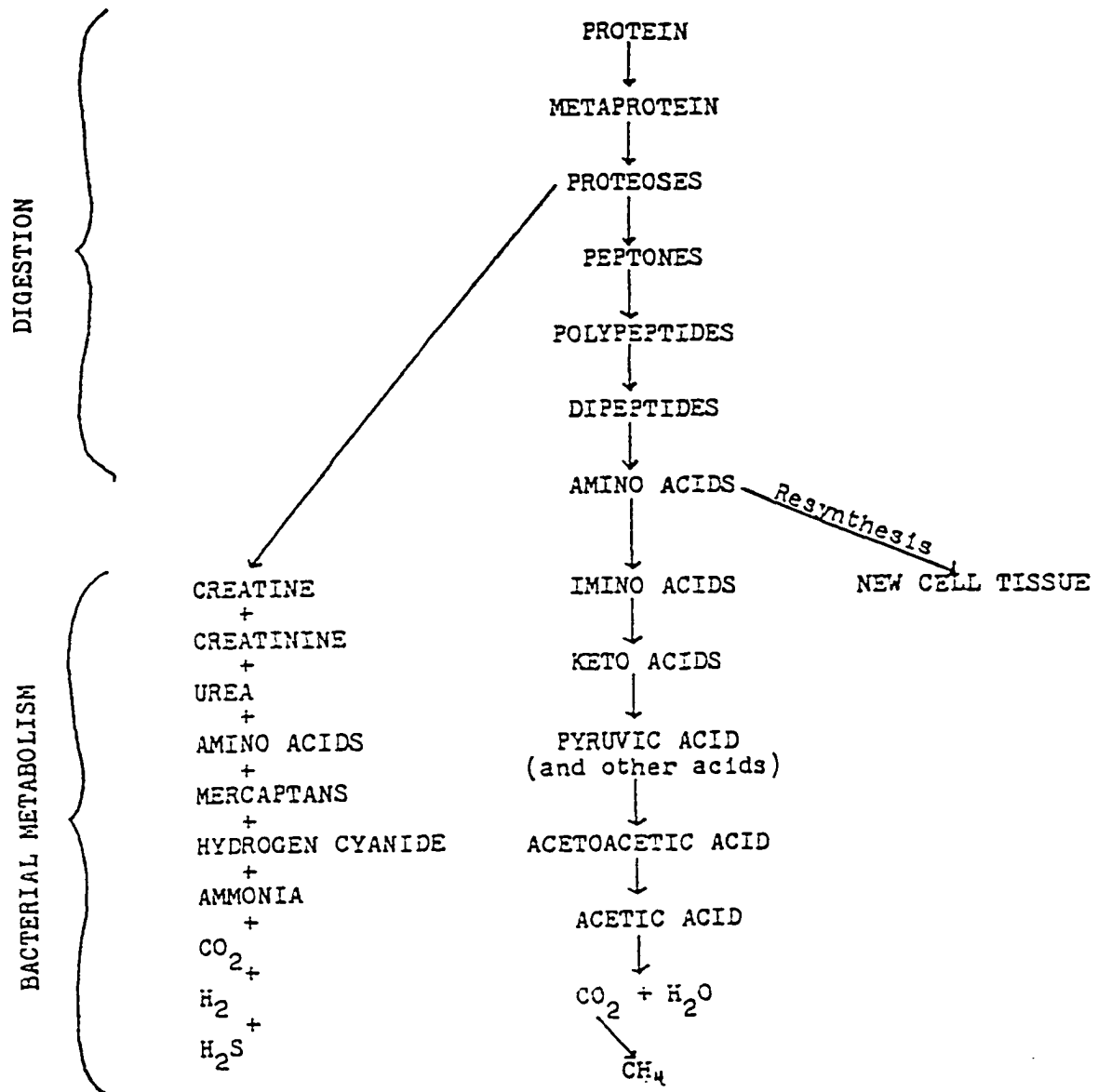


Fig. 2-8. A possible pathway for anaerobic degradation of protein [Chanin, 1961]

Inadequate species diversity is one of the cause of difficulty in process control and failure of anaerobic systems. It is more obvious for the treatment in the unfavorable environmental and operational conditions such as at low temperature and at low substrate concentration. The interspecies interaction is very important for efficient anaerobic treatment system [Grotenhuis et al., 1986].

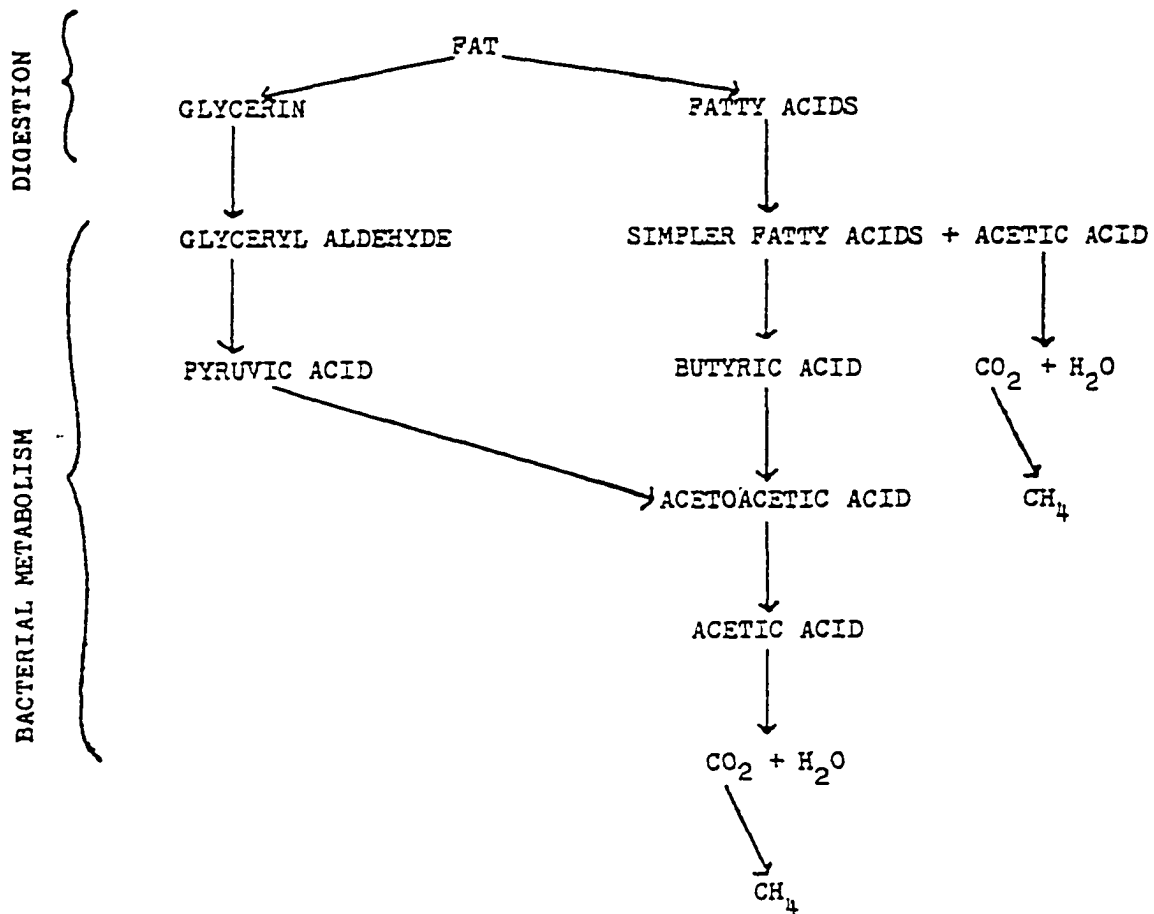
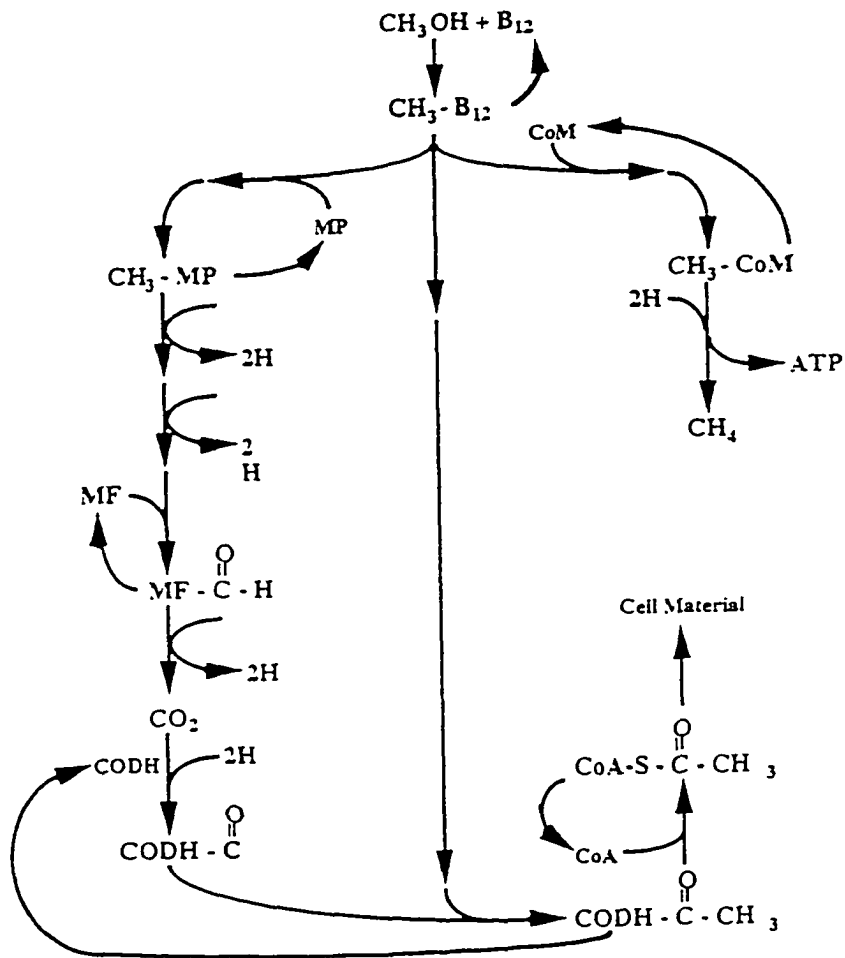


Fig. 2-9. A possible pathway for anaerobic degradation of fat [Chanin, 1961]

There are two instances of substrate competition between groups or classes of bacteria. One example of this is competition between the methanogens and homoacetogens for H_2 and CO_2 . Methanogens convert these compounds to methane, whereas homoacetogens convert them to acetate. In most cases, methanogens are generally able to out-compete the homoacetogens for CO_2 and H_2 .



MF= methanofuran

MP= tetrahydromethanopterin

CoA= coenzyme M

B_{12} = vitamin B_{12}

CODH= carbon monoxide dehydrogenase

Fig. 2-10. Biochemistry of methyl compound utilization by methanogens

Under these conditions, the homoacetogens, which are capable of heterotrophic metabolism as well as autotrophic, utilize organic compounds for carbon and energy [Zehnder, 1988]. A second example of competition for substrates is the well-documented case between the methanogens and the sulfate-reducing bacteria (SRB) [Zehnder, 1988].

Odom et al., 1993]. In the absence of sulfate, some SRB species actually provide substrate (H_2 , acetate) to the methanogens. However, in the presence of sulfate, the SRB generally out-compete the methanogens for H_2 and acetate. This is especially significant at high sulfate and low H_2 or acetate condition. The SRB have lower K_s values for both H_2 and acetate, and, therefore, have a higher affinity for these substrates than do the methanogens, especially under limiting conditions of H_2 or acetate [Zehnder, 1988; Odom et al., 1993].

i. Anaerobic metabolism. The degradation of organic wastes results in the production of electrons which are usually taken by electron acceptors for the completion of anaerobic treatment. Anaerobic respiration occurs when the energy yielding reaction in waste stabilization involves electron acceptors other than oxygen. Some common electron acceptors in anaerobic microbial respiration are presented in Table 2-7.

Table 2-7. Common electron acceptors in anaerobic microbial respiration [Prescott et al., 1990]

Electron acceptor	Reduced products	Microorganisms
NO_3^-	NO_2^- , N_2O , N_2	Pseudomonas and Bacillus
SO_4^{2-}	H_2S	Desulfovibrio and Desulfotomaculum
CO_2	CH_4	All Methanogens
S^0	H_2S	Desulfuromonas and Thermoproteus
Fe^{3+}	Fe^{2+}	Pseudomonas and Bacillus

B. Important Parameters in Anaerobic Treatment

The complexity of anaerobic waste treatment, relative to the microbial consortia and reaction involved, indicate that certain parameters are of significant importance for process control and system stability. The parameters affecting anaerobic treatment can be broadly classified as environmental and operational. The environmental parameters include: temperature, pH, alkalinity, volatile acids, ammonia, sulfate, toxic metals and salts, and inhibitory intermediate products. The operational parameters included solids concentration (MLSS), solids retention time (SRT), food to microorganism ratio (F/M), organic concentration and loading rate, and hydraulic retention time (HRT).

1. Environmental Parameters

a. Temperature. Temperature has a profound effect on the rate as well as the degree of biological waste treatment. Early studies on the effect of temperature in an anaerobic processes were mainly on sludge digestion, and not in liquid waste treatment. Some of these early studies include a report by Rudolfs (1927) who studied environmental digestion at temperatures of 10, 18, 24, 29.5 and 35 °C. He found that the total amount of gas produced from a gram of sludge was not affected by temperature, but that the rate of gas production was affected by temperature. His results showed that more time was required for sludge destruction at lower temperatures in comparison to higher temperatures.

A study by Heukelekian (1933) showed that equal sludge digestion time in a batch process at temperatures of 28 and 42 °C. Present knowledge on biological waste treatment has identified three temperature ranges for waste treatment. These temperatures are

Thermophilic temperature (45°-75°C)

Mesophilic temperature (25°-45°C)

Psychrophilic temperature (<25°C)

It has been generally accepted that the organisms responsible for digestion at thermophilic temperatures are different from those responsible for digestion at lower temperatures (psychrophilic and mesophilic).

The rate of biological treatment has been generally assumed to double for every 10 °C rise, and vice versa, due to the reduction of microbial metabolic rate at lower temperatures. This is called by K-10 rule. The relationship between the reaction rate constant for substrate utilization and temperature for the biological treatment is given by the Streeter-Phelps equation expressed as [Benedict and Carlson, 1970, 1974; Grady and Lim, 1980]:

$$K_2 = K_1 \theta^{T_2 - T_1} \quad 2-1)$$

$$\text{or} \quad K_r = K_{20} \cdot \theta^{T - 20} \quad 2-2)$$

where, K_1, K_2 = Reaction rate constant at the temperatures of T_1 and T_2 respectively

θ = Temperature Coefficient

For many biological systems, θ is equal to 1.07. By applying Equation 2-2 at a temperature of 30 °C, the ratio of K_{30}/K_{20} is equal to 1.97, essentially 2. Therefore Equation 2-2 predicts the biological rates will be twice as fast at 30 °C as they are at 20 °C. However, temperature dependency of reaction rates can be defined by Arrhenious expression [Grady and Lim, 1980]:

$$K = K_0 e^{-(E/RT)} \quad 2-3)$$

where, K = reaction rate constant, mg/l

K_0 = frequency factor, a constant

E = activation energy, cal/mole/°K

R = universal gas constant, cal/mole/°K

T = absolute temperature, °K

In addition, a reduction of treatment temperature affects biogas recovery from the reactor due to a increase of solubility of gases [Metcalf and Eddy, 1991], for the reduction of Henry's law constant. The changes of Henry's law constant with the temperature are shown in Table 2-8.

Table 2-8. Solubility constants of methane and CO₂ at various low temperatures
[Metcalf & Eddy, 1991]

Temperature, °C	H _{CH₄} × 10 ⁻⁴	H _{CO₂} × 10 ⁻⁴
0	2.24	0.0728
5	2.61	0.0884
10	2.97	0.104
15	3.37	0.123
20	3.76	0.142
25	4.12	0.164
30	4.49	0.186

Anaerobic treatment has generally been operated at mesophilic temperature. This usually demands the application of external heating to the reactor. The heat requirement is achieved by direct utilization of the methane gas, produced from stabilization. However, for the treatment of dilute (low strength) wastewater where methane production is low, it is necessary to successfully operate the system at ambient temperatures. High-rate anaerobic treatment of dilute wastewater at low temperature will be reviewed later in this literature review.

Temperature affects the rates at which the digestion is carried out, rather than the total degree of digestion that is achieved. Pidaparti and Dague [1992] reported similar total COD and volatile solids destruction at temperatures of both 35 °C and 25 °C. However, since the

rates of degradation are slower at lower temperatures, the digestion process must be allowed to operate at longer retention times or the biomass population must increase in order to obtain the same degree of stabilization at lower temperatures.

Another important effect of temperature on biological waste treatment is the effect of temperature on the settleability of biomass. The effect of temperature on biomass settleability can be explained in terms of the viscosity of the reactor fluid. Viscosity is used to describe here the fluidity of a fluid to the tendency of a fluid to resist motion. Generally, with the decrease of temperature, the viscosity increase resulting in low settleability.

The effect of fluid viscosity on gravity settling of discrete particles is presented in the well known Stoke's equation [Tchobauoglous and Schroeder, 1985]:

$$V_s = \frac{g(\rho_p - \rho_w)d_p^2}{18\varepsilon} \quad 2-4)$$

where,

V_s = particle settling velocity, m/s

g = acceleration due to gravity, m/s²

ρ_p = density of particle, kg/m³

ρ_w = density of water, kg/m³

d_p = diameter of particle, m

ε = dynamic viscosity of water, kg/m/s

The above equation shows that the settling velocity of a given biomass particle decreases with increases in viscosity of the fluid, with other factors remaining constant. With the decrease of temperature, the viscosity or the resistance of settling velocity increase. For example, as the temperature of water changes from 100 °F to 60 °F(37.7 ° to 15 °C), its viscosity increases by about 40 percent with only one percent increase in density [Munson et al., 1990]. This means that a particle of biomass has about 40 percent resistance to overcome during settling at 15 °C as compared to settling at 37.7 °C. Davis and Cornwell [1991] present properties of water at various temperatures (Table 2-9).

Biomass responses to changes in their environment, for which temperature changes during studies with anaerobic reactors should be slow and gradual to avoid a shock to the system. Dague [1967] used a step-wise temperature change of 1 °C per day to facilitate system stability. Henze and Harremoes [1983] also suggest that temperature changes in anaerobic studies should be done slowly such as a step of 1°C per day. The gradual change of temperature encourage the microorganisms to adapt without halt in metabolic process. although the metabolic rate changes.

Table 2-9. Physical properties of water at 1 atm [Davis and Cornwell, 1991]

Temperature	Density (kg/m ³)	Dynamic Viscosity (m.pa.s)
0	999.842	1.1787
5	999.967	1.519
10	999.703	1.307
15	999.103	1.139
20	998.207	1.002
25	997.048	0.89
30	995.65	0.798

b. Hydrogen ion concentration (pH). The hydrogen ion concentration in an anaerobic treatment system has a major effect on the performance. McCarty [94] suggested a range for pH of 6.6 to 7.6, with an optimal between 7 to 7.2. Other reports have indicated that methanogenesis is possible at pH values as low as 6 to 6.2 and as high as 8.1 to 8.5 [Holland et al. 1987; Seagram et al., 1990; Zehnder, 1988]. Since the methanogens are the most important organisms in an anaerobic system, it is important that the pH be maintained at a level (pH = 7) that is not inhibitory to them. The effect of a non-optimum pH level in anaerobic treatment is shifting of many reactions and equilibrium conditions in the reactor.

The pH of a reactor fluid undergoing anaerobic treatment is related to the acid-base chemical equilibrium. The hydrogen ion concentration is related to the carbonic acid and bicarbonate concentration by the expression:

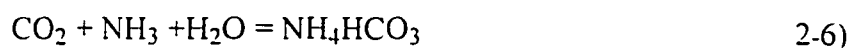
$$[H^+] = K_1[H_2CO_3^*]/[HCO_3^-] \quad 2-5)$$

where, $[H^+]$ = Hydrogen ion concentration, mole/L
 K_1 = Ionized constant for carbonic acid at the given temperature and ionic strength, Mole/L
 $[H_2CO_3^*]$ = Carbonic acid concentration, Mole/L
 $[HCO_3^-]$ = Bicarbonate concentration, Mole/L

Sawyer and McCarty [1978] and Benefield, Jenkins and Weand [1982] defined $[H_2CO_3^*]$ to be equal to the sum of the molar concentration of $[H_2CO_3]$ and $[CO_{2(aq)}]$ due to the fact that it is difficult to distinguish between the two.

Since anaerobic degradation does not occur at pH below 6.6, sufficient alkalinity is required to provide buffering so that pH can maintained at the neutral level.

c. Alkalinity. Alkalinity is the capacity of a solution to neutralize acids, used for maintaining pH at neutral level. In most wastewater applications, the main component of alkalinity is the bicarbonate ion, although carbonate, hydroxyl, phosphate, and borate species also contribute to alkalinity to varying degrees. Bicarbonate is mainly produced by the formation of ammonium bicarbonate from CO_2 and NH_3 during anaerobic degradation of complex substrates (e.g., protein) by the following reaction:



The ammonium bicarbonate dissociates to NH_4^+ and HCO_3^- . The bicarbonate ion provides a buffer to decrease in pH through the carbonic acid/bicarbonate system, as shown in the following equation.



Examination of the Equation 2-7 shows that the carbonate acid-bicarbonate system is directly influenced by the pH of the reactor and vice versa. Therefore, as acid (H^+) is added to the system, bicarbonate is used to produce carbonic acid (which is also in equilibrium with carbon di oxide and water), but the pH of the system will not decrease significantly until the majority of the bicarbonate is gone. Many wastewaters do not contain sufficient alkalinity to maintain proper pH levels over the course of treatment. In these applications, it is usually necessary to add alkalinity to ensure maintenance of appropriate pH. Common alkaline compounds used for this are ammonia, lime, sodium bicarbonate, and sodium hydroxide [Dague, 1981, McCarty, 1964]. A relationship between alkalinity, pH and CO_2 in digester gas is shown in Figure 2-11.

d. Volatile fatty acids (VFA). Volatile acids are produced through the degradation of more complex molecules by the acidogenic and acetogenic bacteria. Usually, a volatile acids increase in an anaerobic system indicates an imbalance in microbial activity and results in the drop of pH value. If the volatile acid (formic, acetic, propionic, butyric, valeric, isovaleric) increase to high level, a new equilibrium establish between the acids and their respective salts. Equation 2-7 represents the H^+ produced by acetic acid ionization.



Since the PK_a of most of the volatile acids is less than 4, most of the acid will be present in the ionized form, thus producing H^+ with a subsequent decrease in pH unless sufficient alkalinity exists to neutralize the acid. A high volatile acids concentration in conjunction with low pH (<6.5) is a prevalent problem with improperly operated anaerobic systems for which it called as "stuck" digester. In order to relive this condition, the pH must be raised to non-inhibitory levels with an alkaline material, often lime. The methanogens may then function properly to remove the acids and produce methane. It has been noted that it is the low pH, rather than high volatile acids concentration, that inhibits proper anaerobic digestion.

Volatile acids concentration is related to the system alkalinity as follows [McCarty, 1964b]:

$$BA = TA - (0.85)(0.833)TVA \quad 2-9)$$

where, TVA = total volatile acid concentration, mg/L as acetic acid
 BA = bicarbonate alkalinity, mg/L as CaCO₃
 TA = total alkalinity, mg/L as CaCO₃
 0.85 = factor that only 85% of the VFA alkalinity is measured by titration
 of TA to pH 4
 0.833 = conversion factor to change TVA in mg/L as acetic to TVA
 alkalinity in mg/L as CaCO₃

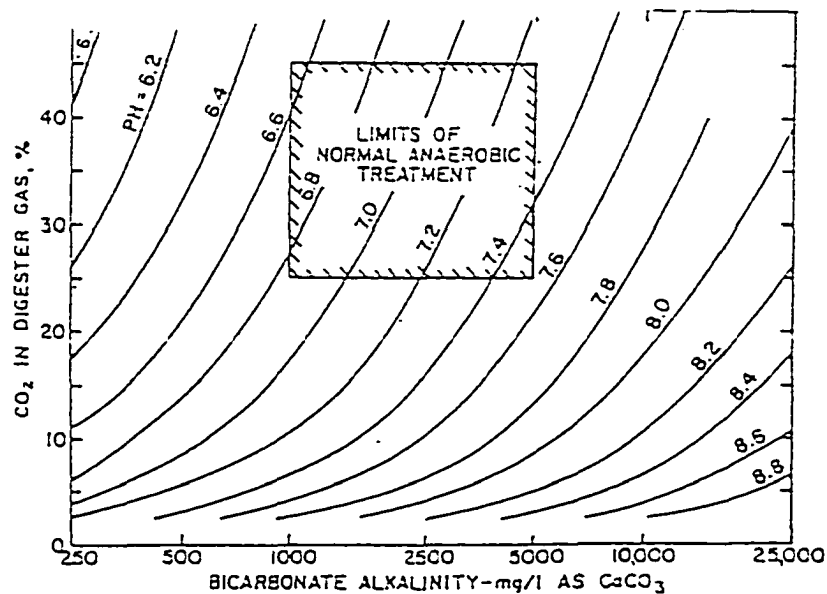


Fig. 2-11. Relationship between pH, CO₂ percentages and [HCO₃] at 95 °F [McCarty, 1964b]

e. Toxic or inhibitory compounds. Many organic and inorganic materials may be toxic to anaerobic organisms. Often, at low concentrations, these same materials may be stimulatory to the microorganisms, but as the concentration of the material increases, it becomes inhibitory and finally toxic. The alkali and alkaline earth metals which include sodium, potassium, calcium and magnesium, are examples of compounds that exhibit this phenomenon [McCarty, 1964; Rinzema et al., 1988]. McCarty [1964] reported on the effects of these materials on anaerobic digestion, which are shown in Table 2-10.

Table 2-10. Stimulatory and inhibitory effects of alkali and alkaline-earth metals on anaerobic digestion [McCarty, 1964]

Cations	Concentrations (mg/L)		
	Stimulatory	Moderately Inhibitory	Strongly Inhibitory
Sodium	100-200	3500-5500	8000
Potassium	200-400	2500-4500	12000
Calcium	100-200	2500-4500	8000
Magnesium	75-150	1000-1500	3000

According to the McCarty [1964c], the possible methods to control toxic materials in anaerobic treatment include:

- remove toxic material from waste
- dilute below toxic threshold
- form insoluble complex or precipitate
- antagonize toxicity with another material

Heavy metals such as zinc, copper, nickel, chromium, lead, cadmium and iron are known to be toxic to anaerobic bacteria at some concentration [Mosey and Hughes, 1975]. Also, most of the requires nutrients are toxic at high concentration. Figure 2-12 shows the effect of salts or other materials on biological reactions [McCarty 1964c].

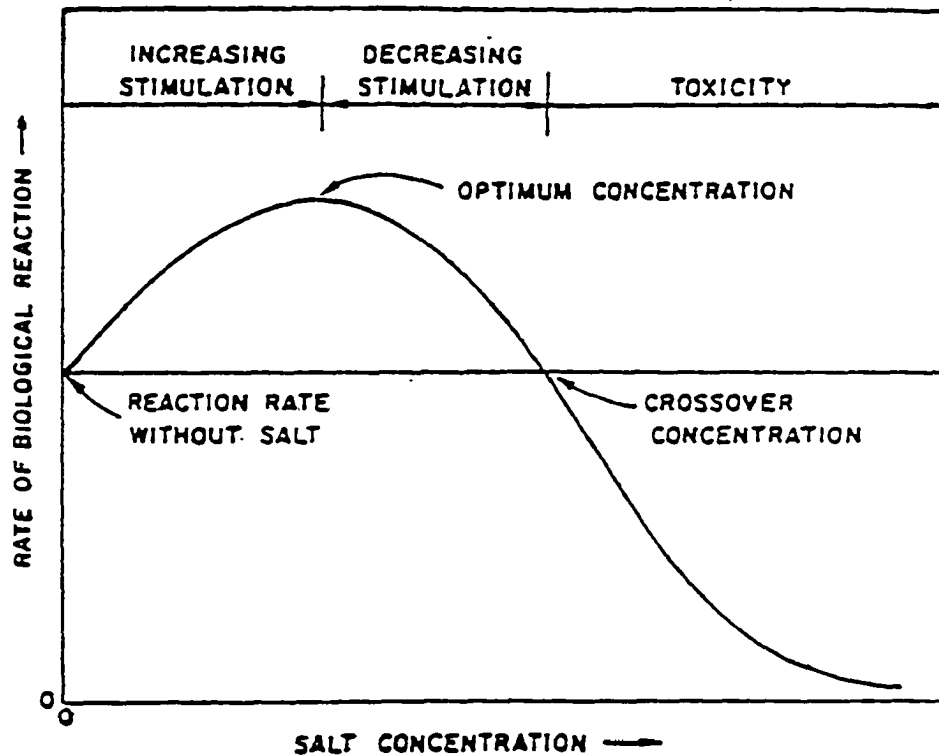


Fig. 2-12. General effect of salts on biological reactions [McCarty, 1964c]

f. Ammonia. Ammonia is another toxic to anaerobic systems. Anaerobic treatment of protein-bearing wastes results in the production of ammonia which reacts with carbon dioxide gas in the reactor to form ammonium bicarbonate (NH_4HCO_3). Ammonia may be present in the form of ammonium ion (NH_4^+) or dissolved ammonia gas (NH_3) [McKinney and McCarty, 1961]. The equilibrium between the two forms is given by:



The equilibrium concentration of the two ammonia species in water as a function of pH is shown in Figure 2-13. At 25°C, the pKa for ammonia system is 9.3 [Sawer and McCarty, 1978]. Therefore, at 25 °C and pH 9.3, half of the ammonia would be in the NH_3 form and the other half in the NH_4^+ form. From the above equation 2-10, the following equation at 35 °C can be drawn [McCarty and McKinney, 1961]

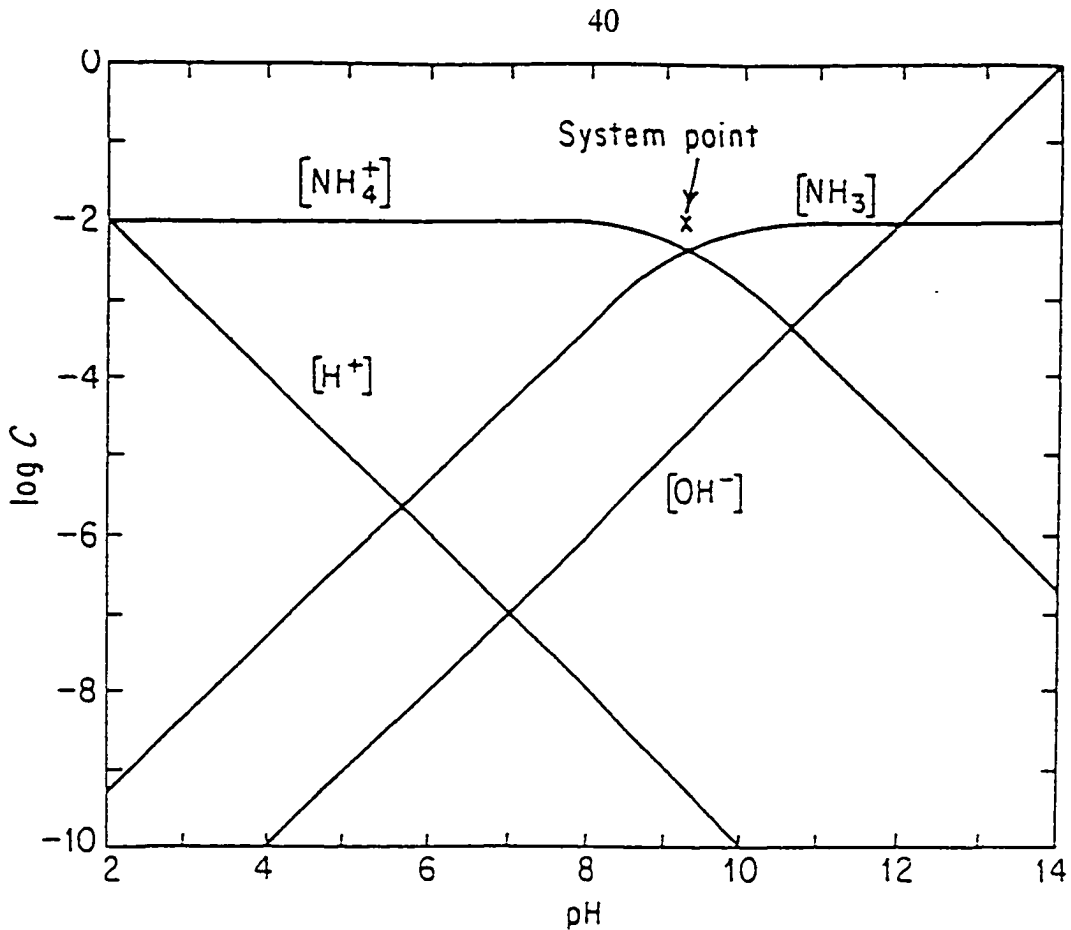


Fig. 2-13. Logarithmic concentration of 0.01 ammonia system [Sawyer and McCarty, 1978]

$$\frac{[NH_4^+][OH^-]}{[NH_3]} = 1.85 \times 10^{-5} = K_b \text{ at } 35^\circ\text{C} \quad 2-11)$$

Ammonia has been found to be both beneficial and inhibitory in anaerobic systems based on the concentration of the total ammonia (NH_3 plus NH_4^+). According to McCarty [1964c], free ammonia is inhibitory at much lower concentrations than the inhibitory concentration for the total ammonia ($NH_3 + NH_4^+$). McCarty and McKinney found that the increase in ammonium ion concentration $[NH_4^+]$ led to a decrease in the rate of substrate utilization while an increase in the concentration of free ammonia led to toxicity and complete halt of the microbial activity. The ammonia concentration and their effects in anaerobic systems are shown in Table 2-11.

Table 2-11. Effect of ammonia nitrogen on anaerobic treatment [McCarty, 1964c]

Ammonia Nitrogen Concentration, mg/L	Effect
50-200	Beneficial
200-1000	No adverse effect
1500-3000	Inhibitory at higher pH values
Above 3000	Toxic

g. Inhibitory treatment intermediates. Some anaerobic treatment intermediate products such as alcohols and organic acids are toxic to anaerobic systems [Herrero, 1983]. As discussed earlier, the accumulation of intermediate products indicates unbalanced trophic relationship between the H₂-producing bacteria and methanogens.

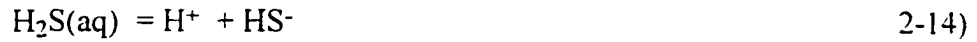
The toxicity caused by the accumulation of alcohol in an anaerobic reactor is known to relate to changes in the structure and function of microbial cellular membranes. For example, the fatty acid composition of C. thermocellum membranes is known to be altered in the presence of ethanol. Briefly speaking, the net effect of excess organic acids in anaerobic systems is the requirement of energy expenditure by biomass to restore equilibrium pH between the interior and the exterior of the cell which increases energization of the cell membrane to a level where inactivity and inhibition occur [Herrero, 1983].

h. Effects of sulfate. In the anaerobic biological treatment system, when sulfate is fed, it usually undergoes biological reduction to sulfide. The reduction of sulfates by the sulfate reducing bacteria (SRB) is given by the equations [Lawrence et al., 1964]:



The sulfide in the anaerobic reactor may be present in a soluble form or insoluble form depending upon the cations with which they are associated. Several of the common metal

sulfides, such as iron, copper, zinc, and nickel, are known to be insoluble, and will precipitate and remove the sulfides from solution while the remaining soluble sulfide form a weak acid which ionizes in aqueous solution, the extent depending upon the pH [Lawrence et al., 1964]. Usually, the soluble sulfide is hydrogen sulfide (H_2S) which results from the reaction of the sulfur with hydrogen ions in the reactor [Metcalf and Eddy, 1991]. The soluble form of sulfides in an anaerobic reactor may include H_2S , HS^- , S^{2-} [Lawrence et al. 1964, 1966]. Lawrence et al. also found that only the first dissociation of hydrogen sulfide (H_2S) is important in anaerobic systems due to the neutral pH at which the systems are usually operated. The dissociation relationship is therefore given by:



The relationship between hydrogen ion concentration and sulfides is given by [Lawrence et al., 1964]

$$[H^+] = K_1[H_2S(aq)]/[HS^-] \quad 2-15)$$

Because of the limited solubility of hydrogen sulfide, a certain portion usually escape with the reactor gas. The resulting equilibrium between hydrogen sulfide remaining in the reactor fluid and that in the gas above the fluid level is governed by Henry's Law as follows:

$$[H_2S(aq)] = \alpha[H_2S(g)] \quad 2-16)$$

where, $[H_2S(aq)]$ and $[H_2S(g)]$ are expressed in units of moles per liter of liquid and mole per liter of gas, respectively. The values for K_1 in equation 2-14 and α in equation 2-15 are given in Table 2-12.

The prediction of the distribution of the sulfides between the gas and aqueous phases is accomplished by considering the relationship between pH and the different forms of soluble sulfide, as well as the solubility of the hydrogen sulfide itself.

$$T.S.S. = [HS^-] + [H_2S(aq)] \quad 2-17)$$

Table 2-12 . Values for sulfide equilibrium constants [Lawrence et al., 1964]

Temperature, °C	Ionization Constant, K_1 $\times 10^8$	Absorption Coefficient, α
18	9.1	2.72
25	11.2	2.28
35	14.9	1.83
45	19.4	1.52

By equating the equations 2-15, 2-16 and 2-17, the found equation is

$$[T.S.S.]/[H_2S(aq)] = \alpha(1 + K_1/H^+) \quad 2-18)$$

where, T.S.S. = total concentration of soluble sulfides

From the equation 2-18, it is possible to calculate the equilibrium ratio for the concentration of soluble sulfides in the reactor supernatant liquid to the concentration of hydrogen sulfide in the gas, knowing the reactor pH and temperature.

Early research experience with the conventional continuous flow stirred tank reactor had shown that high sulfate content in waste stream is toxic to anaerobic reactor due to reduction of sulfate to sulfide. One of the problems associated with the presence of sulfate and sulfate-reduction in anaerobic reactors is the competition between the sulfate-reducing bacteria (SRB) and the methane producing bacteria (MPB). As shown in Table 2-13, sulfate reducing bacteria use a variety of nutrients which include fatty acids, propionate, acetate, CO₂ and organic compounds for energy and cell synthesis with sulfate as their terminal electron acceptor [Lawrence et al. 1964]. Methanogens also use same type of substrate. The competition between the methanogens and the sulfate-reducing bacteria in anaerobic environment have been studied by many researchers, such as Isa and others [1986a, 1986b], Khan and Trottier [1978], Robinson and Tiedje [1984], Ueki and others [1986], and Van den

Berg and others [1980]. The competition between the SRB and MPB for the same substrate usually results in an upset of the reactor, especially when sulfide concentration exceeds 200 mg/L. McCarty [1964c] also reviewed sulfide toxicity in anaerobic treatment. He stated that "concentration of soluble sulfide varying from 50 to 100 mg/L can be tolerated in anaerobic treatment with little or no acclimation required. That with continuous operation, concentration up to 200 mg/L of soluble sulfides can be tolerated with no significant inhibitory effect on anaerobic treatment". He added that "concentration above 200 mg/L are quite toxic and toxic concentration of sulfide may be reduced by gas scrubbing, use of iron salts to precipitate sulfides, dilution of waste, or separation of sulfate or other sulfur containing streams from the waste to be treated." (p.93-94)

In summary, the presence of sulfate reducing bacteria (SRB) in anaerobically treated wastewater is a problem because: 1) SRB will compete with methanogens for the same organic matter as the source of energy; 2) sulfate is reduced to sulfide in the anaerobic

Table 2-13. Sulfate -reducing bacteria species [Hamilton, 1983]

Gram					
Species	Reaction	Morphology	Nutrition	Biological Reactions	
Desulfovibrio	-	Cirved rod	Fatty acids(C18)	→ acetate	$\text{SO}_4^{2-} \rightarrow \text{S}^{2-}$
sapovorans				propionate	
Desulfobulus	-	Lemon shaped	Propionate	→ acetate	$\text{SO}_4^{2-} \rightarrow \text{S}^{2-}$
propionicus					$\text{NO}_3^- \rightarrow \text{NO}_2^-$
Desufotomaculum		Spore-forming	Acetate	→ CO_2	$\text{SO}_4^{2-} \rightarrow \text{S}^{2-}$
acetoxidans		rod			
Desufobacter		Rod to	Acetate	→ CO_2	$\text{SO}_4^{2-} \rightarrow \text{S}^{2-}$
		elliptical			
Desulfonema	+	Filaments	Organic	→ CO_2	$\text{SO}_4^{2-} \rightarrow \text{S}^{2-}$
magnum		(7 μm dia.)			

environment, which can cause the precipitation of nutrients and metals necessary for methanogens; and 3) un-ionized hydrogen sulfide is toxic to MPB [Parkin et al., 1991].

An important parameter in anaerobic treatment of sulfate bearing wastewater is the ratio of organic matter and sulfate content of the wastewater. Choi and Rim [1992] studied the competition and inhibition of sulfate reducers and methane producers in anaerobic treatment using laboratory contact units. The systems were operated at 35 °C and hydraulic retention times (HRT) ranging from 0.5 to 6 days. A synthetic substrate (acetic acids) supplemented with nutrients was used as the wastewater. The COD of the wastewater was fixed at 2000 mg/L. Although no source of sulfate was given, the sulfate content of the wastewater was adjusted to provide COD/sulfate ratios ranging from 0.4 to 13.3. In addition, they treated sea food waste and glutamic acid waste which had COD/sulfate ratios of 1.7 and 1.0 to 1.2 respectively. They found that sulfate reducers began to predominate at COD/sulfate ratio of less than 1.7, with full domination at the ratio of 0.4. They also found that sulfate reducers and methane producers were very competitive at COD/sulfate ratios of 1.7 to 2.7, with methane producers dominating at COD/sulfate ratio of 2.7 and above. In terms of sulfate concentration, their results showed that 1200 mg/L sulfate (or sulfide 120 to 140 mg/L) was inhibitory to methane producers while a concentration of 2000 mg/L sulfate (or sulfide 160 to 200 mg/L) was inhibitory to sulfate reducers. Their results also showed that the inhibitory concentration of sulfate was dependent upon the type of waste as 240 mg/L sulfide did not inhibit methane production in sea-food waste treatment.

The use of ferric chloride to precipitate sulfide has been reported to be an effective means for successful anaerobic treatment of sulfate bearing wastewater [Choi and Rim, 1991]. However, there is a concern that such precipitation may also remove necessary nutrients from the wastewater resulting in poor treatment systems. Other researchers [Clancy et al., 1992; Lo et al., 1990] have reported on Molybdate inhibition of sulfate reduction in

anaerobic reactors. They report that Molybdate has the ability to deplete the ATP in sulfate reducing bacteria and renders them incapable to reduce sulfate to sulfide.

Reports in the literature show that unlike the conventional CSTRs, high-rate anaerobic reactors are capable of treating wastewaters with high sulfate contents [Isa et al., 1986a, 1986b, Callado et al., Undated]. Studies by Callado et al. investigated treatment of sulfate bearing wastewater in the upflow anaerobic sludge blanket (UASB) reactor. The treatment study utilized a 10 L laboratory scale UASB, a 2000 mg/L COD synthetic wastewater and Na_2S solution as the source of sulfate. The reactor was operated at HRT of 15.6 hrs. Sulfate solution was added to the wastewater at concentrations of 25, 50, 100, 150, 200, 300, 400, 500, 750, 1000, 1250, 1500, 1750, 2000, 3000, 4000, 5000, 7500, and 10000 mg/L as sulfate resulting in COD/sulfate ratio ranging from 0.2 to 80. After seeding, the reactor was allowed to reach 95% COD removal prior to a step increase in sulfate concentration. They reported that the results did not show any severe effects due to the step increase in sulfate concentration. Their results showed COD removals of above 95% at low sulfate concentration and above 80% at sulfate concentration of 10000 mg/L (COD/sulfate ratio of 0.2) based on centrifuged effluent. They also reported that the UASB reactor was characterized by a gradual assimilation of the shock load and that with the exception of the very high values of influent sulfate concentration, the COD removal efficiency was always restored at more than 85%. They state that similar high efficient treatment of sulfate bearing wastewater has also been reported to occur in the anaerobic fluidized bed reactor.

Isa et al. [1986b] reported on high-rate anaerobic treatment of sulfate bearing wastewater using a one liter laboratory scale reactor which was operated at 35 °C and at organic loading rate of 10 gCOD/L/day. Synthetic substrates (acetate and acetate plus ethanol) supplemented with mineral nutrients were used as the wastewaters. Sulfide was added by dissolving $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ to obtain 20 g of sulfate as S per liter. Both wastewaters were treated at HRTs of 12 hrs and 10 days. Their results showed 27% and 35% sulfate

reduction at 12 hr and 10 days HRT respectively. That is, their results showed decreasing sulfate reduction with decreasing HRT. Parkin et al. [1991] also reported on successful anaerobic treatment of sulfate bearing wastewater using the anaerobic filter. They reported that the levels of sulfide in the reactor which resulted in a decrease in performance were significantly higher than those levels causing inhibition in the conventional complex mix anaerobic reactors.

2. Operational parameters

a. Introduction. Three important parameters include solid retention time (SRT), hydraulic retention time (HRT), and organic loading rate are the operational parameters under direct control of operator. Control of these parameters defines the loading criteria of the anaerobic system.

b. Solid retention time. The solid retention time (SRT) of an anaerobic system is the average time that a solid particle is retained in the reactor. If the SRT is less than the microbial regeneration time of the slowest growing organism, the proper consortium of anaerobic bacteria may not be maintained in the system [Dague et al., 1970]. Since the slowest growing anaerobes are the methanogens, SRT control is especially beneficial to these organisms. The SRT of a system is defined as the mass of solids in a system divided by the mass of solids lost from the system per time. If the system is in equilibrium condition, the SRT may also be defined as the mass of the solids in the system divided by the mass of solids produced per time, since the mass produced is equal to the mass lost.

The loss of biomass with the effluent and production of biomass is important both to the accumulation of mixed liquor suspended solids (MLSS) in the system, maintenance of long solids retention time, and the overall reactor performance. Excess biomass in the effluent results in less MLSS and low SRTs. If the SRT is less than the regeneration time of the microorganisms, resulting in imbalance of microbial species and the required cooperative

"community" of microorganisms, especially the methane-formers. This, in turn, results in an incomplete conversion of the substrate to methane and carbon dioxide, accumulation of VFA and inefficient system.

For aerobic system, microbial degradation of substrates such as sugars, alcohols, lipids or protein is said to result in about 0.4 g cell dry weight per gram of COD removed [Verstraete and Vaerenbergh, 1986]. However, Jewell [1987] states that more than 50% of the substrate removed by aerobic system is converted to new biomass as compared to less than 15% of organic conversion to new cells in anaerobic systems.

Generally, the amount of biological solids produced during waste treatment depends, in part, on the strength of waste and organic loadings. Therefore, a concentrated wastewater will result in high levels of biological solids production. Because less biological solids are produced during the treatment of low strength wastewater, it is important that excessive loss of suspended solid (SS) does not occur.

The required SRT is dependent on the temperature of the system since, as temperature increases, microbial regeneration times decreases. Therefore, at high temperatures, the required SRT is less than that required at lower temperatures. Dague et al.[1970] reported that the required SRT for stable anaerobic treatment at 35 °C is approximately 10 days. Because of increased metabolic rates at higher temperatures, the minimum SRT at a temperature of 55 °C is only 2 to 3 days. But for the low temperature such as 15 °C, SRT should be much higher than 10 days. McCarty [1964d] presented the minimum and suggested SRT for anaerobic treatment for different temperatures. He states that efficiency of treatment is low and process dependability is poor at minimum SRT. His suggested SRTs for different temperatures are presented in Table 2-14.

Mixed liquor volatile suspended solids (MLVSS) is usually adopted as an estimate of active microorganism although such estimation has some drawbacks especially when the substrate entering the reactor has suspended solids itself [Switzenbaum et al., 1990].

Table 2-14. Suggested solids retention time for the anaerobic system [McCarty, 1964d]

Operating Temperatures, °C	SRT, days
41	10
35	10
29	14
24	20
18.8	28

c. Hydraulic retention time (HRT). The hydraulic retention time (HRT) is the average amount of time that a molecule of water is retained within the reactor. In the case of completely-mixed reactor, the HRT is equal to the SRT. The HRT is important as it affects the organic loading rate. From a cost perspective, it is advantageous to operate an anaerobic treatment system for a given wastewater at the minimum possible HRT. This results in a smaller reactor volume and less capital cost. Typical HRTs for municipal digestors range from 15 to 40 days. High-rate anaerobic processes are often operated at HRTs of 48, 24, 12, or even 6 hrs. HRTs less than 3 hours have been reported for different anaerobic systems [Droste et al., 1987; Schmidt et al., 1997; Kato et al. 1994] for different wastewater.

The HRT also affects the SRT of the system to an extent. If the biomass inventory of a system is the same for two different HRTs, and concentration of biomass in the effluent from the system is also constant, then the low HRT system will operate at a lower SRT of the long-HRT system. This is because more liquid passes through the short HRT reactor over a given time period, and thus, more solids will be carried out of the system, everything else being equal. For a suspended-growth system in which the solids inventory and effluent solids can be measured directly, the SRT can be calculated in terms of solid inventory, effluent solids, and HRT in the equation:

$$\text{SRT} = \text{MLSS} (\text{HRT}) / \text{TSS}_{\text{eff}} \quad 2-19)$$

where, SRT = solids retention time, day
 MLSS = mixed liquor suspended solids, mass/volume
 TSS_{eff} = effluent total suspended solids, mass/volume
 HRT = hydraulic retention time, day

d. Organic loading rate (OLR). The fundamental of microbiology and biokinetics show that the amount and type of food (substrate) plays an important role in the mass and rate of microbial growth. Figure 2-14a shows that when microbial growth is limited by low concentration of required nutrients, the final net growth or yield of cell increases with the initial amount of the limited nutrient present. Figure 2-14b shows that the rate of growth also increases with nutrient concentration, but in a hyperbolic manner. The shape of Figure 14b reflects the rate of nutrient uptake by microbial transport proteins. That is, at sufficiently high nutrient levels the transport systems are saturated, and the growth rate does not rise further with increasing nutrient concentration [Prescott et al., 1990]. The important point about Figure 14a in relation to the treatment of low strength wastewater is that, the total growth of biomass is less at low substrate concentration than at high substrate concentration. Secondly, the rate of microbial metabolism is low at low substrate concentration due to poor contact between the biomass and substrate.

Generally, low organic concentration in wastewater, is associated with poor diffusion of organic matter into the cell walls of bacteria resulting in low levels of organic removals. Therefore, an effective anaerobic treatment system for low-strength wastewater is one which can retain high biomass population. The high biomass population foster close proximity between the substrate and the biomass resulting in efficient treatment. The reactor systems which can retain high biomass, are high-rate biological system.

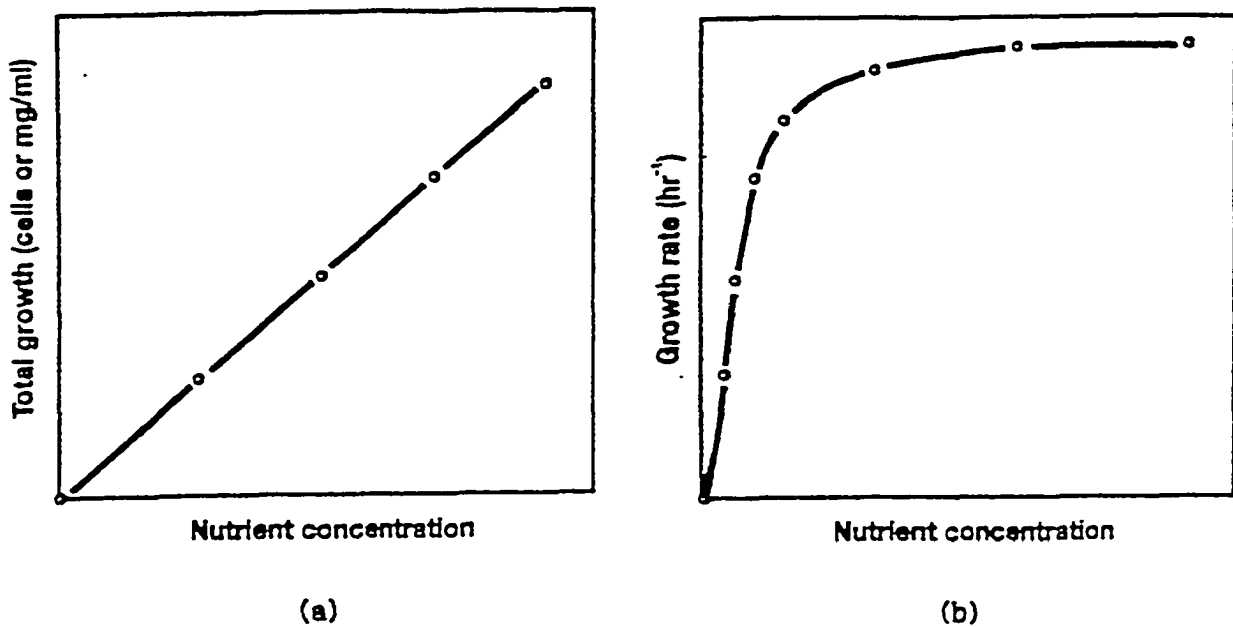


Fig. 2-14. Effect of nutrient concentration on growth of microbes. a) Effect on total microbial yield; b) effect on growth rate [Prescott et al., 1990]

Low substrate concentration is also associated with low gas production due to low organic matter content available for microbial metabolism and subsequent conversion to biogas. This resulting in low natural mixing, avoiding the turbulence of mixing due to the high gas production.

In addition, temperature affect the solubility of methane and carbon dioxide. With the temperature decrease, the solubility of gas increase due to the change of Henry's constant. Sanz and Fdz-Polanco [1989] reported that a 33% loss of methane when treating a 400 mg/L COD wastewater and 60% loss when treating a 200 mg/L COD wastewater due to the solubility of methane at 20 °C. This findings shows low levels of available methane from the treatment of low strength wastewater, especially at lower temperatures and the need for efficient reactors which can operate at ambient temperatures without the requirement for external heating.

The rate at which the substrate enters a reactor is known as the organic loading rate (OLR). This is actually the substrate concentration divided by the HRT and is expressed in grams of substrate (COD or BOD) per liter of reactor volume per day (g/L/day). Therefore, organic loading rate is the mass of pollutant that is pushed through one liter of reactor volume per day. For a low strength wastewater, a short HRT system is required to increase pollutants' load pushed through a reactor.

Although the OLR is a rate at which the substrate enters the reactor, this rate may be different from the rate at which microorganisms are in contact with the substrate. This condition occurs when a reactor is poorly mixed which leads to the development of pockets of substrate where some parts of the reactor see a lot of substrate and the other part see very little or no substrate.

Another importance of substrate concentration in biological treatment relates to the substrate removal efficiency. In the absence of exogenous oxidants such as O_2 , NO_3^- and SO_4^{2-} , the COD of the substrate entering the reactor is the same as COD leaving the reactor. But the exit COD is composed of COD in the effluent, COD converted to the new cells, COD utilized by SRB, and COD converted to methane and CO_2 .

The effect of substrate concentration on substrate removal can be explained by the fundamental equations of substrate utilization in a biological reactor. According to the Lawrence and McCarty [1970], there are two fundamental equations which adequately describe anaerobic biological treatment. The first equation is Monod expression, describes the rate of substrate utilization which is

$$\frac{dS}{dt} = \frac{\mu_{max}}{Y} \cdot \frac{SX}{K_s + S} = \frac{KSX}{K_s + S} \quad 2-20)$$

where,

$$\frac{dS}{dt} = \text{substrate utilization rate, mg/L/time}$$

μ_{\max} = maximum specific growth rate, 1/time

S = substrate concentration, mg/L

K_s = saturation constant (dS/dt/X), mg/L

X = active biomass concentration, mg/L

K = μ_{\max}/Y = maximum specific substrate utilization rate, 1/time

Y = yield coefficient (mg of biomass/mg of substrate utilization)

The equation 2-20 can be reduced to the following equations:

$$\frac{dS}{dt} = \frac{\mu_{\max} X}{Y} \text{ if } S \gg K_s \quad 2-21)$$

$$\frac{dS}{dt} = \frac{\mu_{\max} \cdot X \cdot S}{K_s} \text{ if } K_s \gg S \quad 2-22)$$

That means for the low substrate concentration, the equation 2-21 dominate. The relationship between the substrate concentration and substrate removal rate is shown in Figure 2-15a.

Figure 2-15 revealed that the substrate removal rate is low at low substrate concentration.

The second equation describes the growth of microorganisms which is:

$$\frac{dX}{dt} = \frac{Y \cdot (dS)}{dt} - K_d \cdot X \quad 2-23)$$

where, $\frac{dX}{dt}$ = biomass growth rate, mg/L/time
 $\frac{dS}{dt}$ = substrate utilization rate, mg/L/time

X = biomass concentration, mg/L

Y = biomass yield, mg/mg

K_d = biomass endogenous rate, 1/time

μ = specific growth rate, 1/time

By combining the equations 2-20 to 2-23, the following equation results

$$\mu = Y \cdot \frac{K_s}{K_s + S} - K_d = \frac{\mu_{\max} S}{K_s + S} = \frac{1}{SRT} \quad 2-24)$$

The equation 2-23 can be represented by Figure 2-12. From Figure 2-15b, it is evident that biomass growth is depend on substrate concentration, and low at low concentration. From the above expression 2-20 to 2-24, it can be concluded that the kinetic coefficients (K , K_s , K_d , Y , μ) varies with substrate concentration and temperatures.

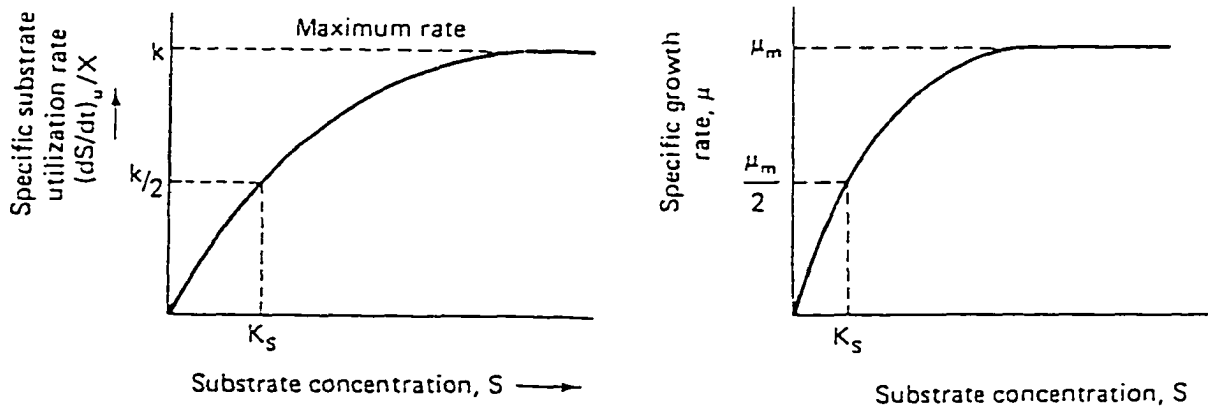


Fig. 2-15. Effect of substrate concentration on a) substrate utilization rate, b) specific microbial growth rate [Benefield et al, 1980]

e. Food to microorganisms (F/M) ratio. Food to microorganism (F/M) ratio is the substrate load applied to the reactor per unit of biomass in the reactor. High F/M ratio means that the MLVSS are saturated with food which results in poor efficiency. A low F/M ratio results in organisms (MLVSS) that are starved and therefore results in complete degradation of wastes. Davis and Cornwell [1991] pointed out that a high F/M ratio corresponds to a short

SRT while a low F/M ratio corresponds to a long SRT. They also indicated that F/M values typically range from 0.1 to 1.0 for efficient treatment.

The importance of F/M ratio in anaerobic treatment was reported by Dague et al. [1966, 1967]. They reported that flocculation of biomass and settleability is achieved at low F/M ratios. Metcalf and Eddy [1991] explained F/M ratio by:

$$F/M = S_o / (\theta \cdot X) \quad 2-25)$$

where, F/M = Food to microorganism ratio, 1/day

S_o = Influent BOD or COD concentration, mg/L

θ = Hydraulic retention time, day (V/Q)

V = Reactor volume, L

X = Concentration of MLVSS, mg/L

Q = Influent wastewater flowrate, L/d

C. Anaerobic Sequencing Batch Reactor (ASBR)

1. Background

A relatively new high-rate anaerobic process, named as Anaerobic Sequencing Batch Reactor (ASBR), was developed in the late 1980s in the environmental engineering laboratories at Iowa State University under the direction of Dr. Richard Dague (Figure 16). Initial research on the ASBR dates back to the 1960s at which time Dr. Dague conducted research on "anaerobic activated sludge" as part of his doctoral research [Dague, 1966, 1967]. At that time, it was recognized that the process could achieve long SRTs while maintaining a low HRT due to internal clarification of the biomass prior to decanting. Some twenty years later, the concept of the anaerobic batch reactor was revisited, resulting several preliminary studies on the ASBR [Dague et al., 1991, 1992; Habben et al., 1991; Sung et al. 1995; Wirtz et al., 1996].

2. Operating Principles

The operating principle of ASBR are simple. The ASBR is fed during a discrete period of time and then operated as a batch reactor. After a desired reaction time, the mixed liquor is allowed to settle and the clarified supernatant is decanted from the reactor. The decant is the treated effluent. As illustrated in Figure 2-16, the reactor sequences through four steps in a complete cycle: feed, react, settle, and decant.

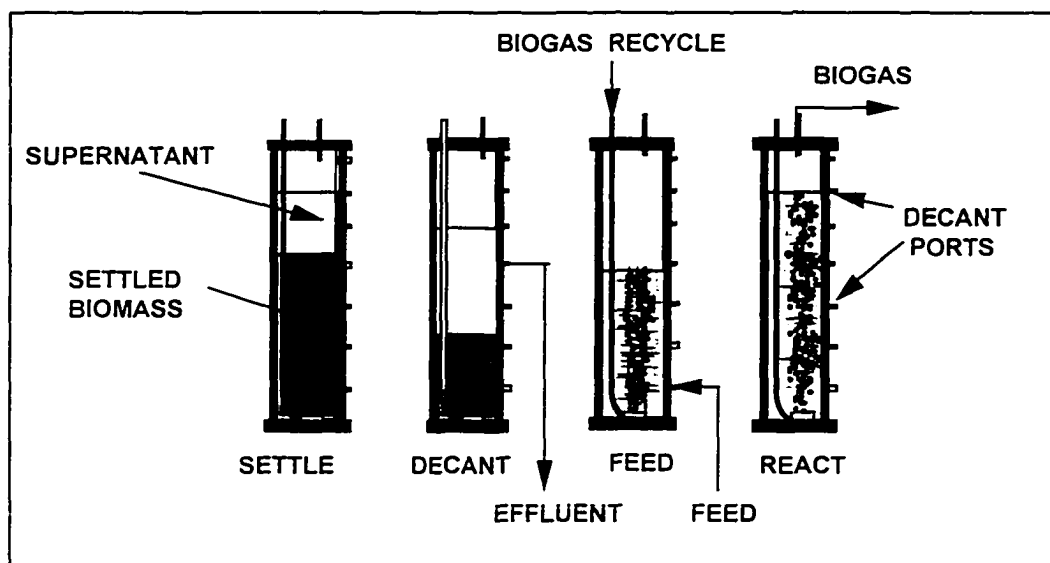


Fig. 2-16. Operating Principle of ASBR.

The feed step involves the addition of substrate to the reactor. The feed volume is normally equal to the volume decanted during the previous decant step (the effluent). With mixing during feeding, the substrate concentration increases rapidly and metabolic rates increase to their highest level. Mixing can be implemented by a motor drive impeller, a hydraulic circulation pump, or a biogas recirculation pump with a bubble diffuser. The feed volume is determined on the basis of a number of factors, including the desired HRT, organic loading, and expected settling characteristics of the biomass.

The reaction step is very important in the conversion of organic substances to biogas. To provide intimate contact between substrate and biomass, mixing is required in the react step. Mixing intensity and duration are preferably operated at minimums so that substrate removal and biomass flocculation or granulation are not adversely affected. The time react for the react step depends on several parameters, including substrate characteristics, required effluent quality, biomass concentration, and waste temperature.

During the settle step, mixing is shut off to enable an ideal quiescent settling condition that allows biomass to flocculate and settle. The reactor itself acts as the clarifier. The time required for the clarification varies, depending on biomass settleability. From an operational point of view, it is essential that the sludge blanket would be below than a predetermined decanting elevation and that the not rise due to the accumulation of biogas within the settled biomass. As discussed earlier, temperature, substrate concentration, the concentration and particle sizes of mixed liquor suspended solids (MLSS) in the reactor are important variables affecting the settling of the biomass and the ability to achieve a clear supernatant for discharge as effluent. An important related variable is the specific process loading rate (food to microorganism ratio, F/M).

The decant step takes place after sufficient solid separation has occurred. The decanting mechanism can be fixed at a predetermined level with the flow regulated valve or a pump, or decant may be adjustable or floating weir just beneath the liquid surface. The time required for the decant step is governed by the total volume to be decanted during each cycle and the decanting rate. Once the decant rate is completed, the reactor is ready to be fed another batch of substrate.

3. Process Description

The ASBR is a non-steady-state, high rate anaerobic treatment. By definition of non-steady-state, the system substrate conversion rate and biogas production rate vary during the cycle. The substrate concentration and liquor volume in the reactor increase during the feed

step from the lowest level at the beginning to the highest level at the end. In the next step, react step, the liquor volume remains constant and substrate concentration decreases as a function of the react time. The react step in the ASBR is a true batch reactor in which the substrate concentration can be simulated by the batch reactor model.

The unique nature of the ASBR system offers different characteristics as compared to the conventional continuous flow system. Mixed liquor solids are not washed out by hydraulic surges since mixed liquor can be held in the reactor as long as necessary. No short circulation occurs in the reactor and no solids or liquid recycle is required. An external clarifier is not required, since biomass separation occurs internally, and saving capital cost. The time-oriented nature of the ASBR provides the freedom of changing the operating conditions by simply resettling the feed or decant volume, or resetting the sequence time. The flexibility is not possible in a special-oriented flow system.

4. Anaerobic Bioflocculation and Granulation

Dague and co-authors (1966) reported that anaerobic biomass was observed to flocculate in a manner not unlike aerobic activated sludge and that food to microorganism (F/M) ratio was an important parameter affecting anaerobic bioflocculation. At low F/M ratios, the biomass flocculated well and settled rapidly, providing a reactor effluent low in suspended solids. Although not recognized at the time (1966), bioflocculation was actually "granulation" in present time (1990). A low F/M ratio can be obtained in one or combination of two ways-lower the food concentration (F) and/or increase the biomass concentration(M).

In a continuously-fed, complete mixed reactor operating at steady-state, the food concentration surrounding the microorganisms is constant. In contrast, in a batch-fed reactor the food concentration is high immediately after feeding and declines until the reactor is fed again, as illustrated in Figure 2-17. The food concentration just prior to feeding is lower in the batch-fed system than at any time in a continuously-fed system. Thus a batch system is

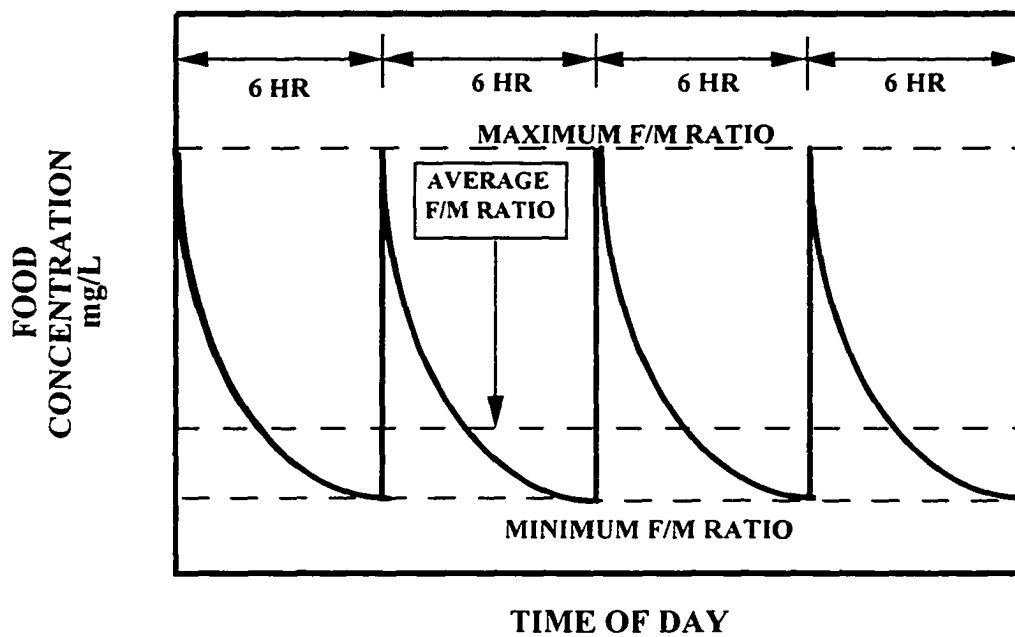


Fig. 2-17. The effect of batch feeding on substrate concentration.

capable of achieving more effluent biomass flocculation and solids separation than is possible in a continuously-fed system. This phenomenon is one of the key characteristics of the ASBR process.

At any given mixed liquor suspended solids concentration in the reactor, the substrate concentration is high immediately after the feed step is completed. This provides a high driving force for metabolic activity and high overall rates of wastes conversion to methane, in accordance with Monod kinetics. Near the end of the react step, the substrate concentration is at its lowest level, resulting in a low gas production rate and providing ideal conditions for biomass flocculation and separation during the settle step.

An important feature of the ASBR is the gradual conversion of the flocculant biomass into well settling and highly active granular biomass (Wirtz et al., 1996). The granulation process can be noticed as the anaerobic microorganisms tend to adhere to one another, as well as to inorganic and/or organic support particles to form firm, dense granules. The ASBR tends to promote the granulation process by imposing a selection pressure during the decant

step. The decanting process tend to wash out the poorly settling flocs and dispersed organisms, and selects for the heavier, more rapidly settling aggregates. Thus, over time, granular biomass becomes dominant and leads to a rapidly settling biomass and a highly stable reactor system.

5. Research on the ASBR

The first study deviated to the ASBR process was conducted by Habben and Dague in the later 1980s and in the early 1990s [Habben, 1991]. The ASBR of this study had liquid volumes of 13 liters and were fed a synthetic substrate consisting of non-fat dry milk plus appropriate nutrients and trace elements. HRTs of 0.54, 1.08 and 2.17 days and COD loadings ranging from 0.5 to 5 g/l/day were investigated at 35 °C. This initial system was capable of 80% COD removal (at the two longer HRTs) when the COD load was 3 g/L/day or less. Higher COD loads resulted in decreased efficiency and failure of the system at 5 gCOD/L/day.

Pidaparti [1991] and Schemidt [1992], reported on the ASBR treatment of swine waste at 35, 25 and 20 °C. It was found that at 35 °C (HRT= 6 days), volatile solids (VS) loading rates of 1.09 to 5.38 g/L/day were investigated, with the volatile solid destruction ranging from 87 to 74%, respectively. At 25 °C(HRT= 6 day), VS loading rates of 1.04 to 6.82 g/L/day were treated VS destruction efficiencies of 92 to 77.6%, respectively. At 20 °C (HRT=6, 9 and 12 days)the VS destruction decreased to a some extent. At VS loading of 0.9 to 5.4 g/L/day at 20 °C, VS destruction ranged from 40 to 70%, but was generally about 50% at VS loading rates greater than 2 g/L/day. Yulin [1996] studied the same waste with the HRTs of 3, 2 and 1 days at room temperatures (22-25 °C), which resulted good performance.

Kaiser et al.[1993] studied the ASBR treatment of non-fat dry milk at thermophilic temperatures (55 °C). The writer demonstrated the capability of the ASBR to achieve stable treatment at COD loadings as high as 9g/L/day at HRTs of 1, 2, and 5 days. This compared to only 3 or 4g COD/L/day for the mesophilic ASBRs of Habben [1991], indicating that much

higher organic loading rates may be possible at higher temperatures. It was also noted that the mixed liquor concentration in the thermophilic ASBRs could not be increased to high levels as is the case for mesophilic ASBRs. This phenomenon was mainly attributed to the increased endogenous decay rates at the higher temperature. Although the biomass growth rate also increases at the higher temperature, it is probable that the increased COD loads possible in the thermophilic ASBRs was not sufficient to sustain enough growth of the biomass to overcome the increased endogenous decay rates.

Herum [1993] studied the effect of applying a vacuum (six inches of water head) to the headspace of mesophilic ASBRs during the final mixing cycle, just prior to the settle phase. It was hypothesized that removal of attached biogas from the biomass would enhance settling, thereby resulting in higher MLSS levels and COD removal efficiencies. The vacuum, applied to the ASBR was found to significantly improve the sludge settleability, with a resulting decrease in biomass lost in the effluent from the reactor, leads to longer SRT. The increased biomass concentrations allowed for better COD removal efficiencies at all COD loading rates, but especially improved the performance of the ASBR at the higher COD loading rates (upto 10 g/L/day). The higher MLSS also provided better recovery from shock loads.

Ndon and Dague [1994] reported on the treatment of dilute wastewater over a range of temperatures. Non-fat dry milk with BOD₅ concentration of 200 to 500 mg/L was treated at temperatures of 35, 25, 20 and 15 °C. Four ASBRs were operated independently at HRTs of 12, 16, 24, 48 hours. Soluble COD removal were generally in excess of 80% under all conditions and generally greater than 90% at the longer HRTs and higher temperatures.

The ASBR has also been applied to several industrial wastewaters with promising results. Tormanen [1993] experienced with mesophilic ASBRs treating a high-sodium search wastewater. Sodium levels averaged 4000 mg/L and were not shown to be inhibitory to the process. Tormann's system employed three ASBRs, two of which were seeded with anaerobic

digester sludge and one that was seeded with granular sludge. HRTs ranged from 18 hrs to 3 days, which correspond to COD loadings of approximately 6 to 1.7 g/L/day, respectively. Efficient treatment (80 to 90% total COD removal) was reported for all three ASBRs at the majority of loading rates.

Another "real-world" waste to which the ASBR has been applied is a landfill leachate from the municipal landfill of Iowa City, Iowa. Two mesophilic ASBRs were used in this study, one seeded with non-granular anaerobic digester sludge and the other with granular sludge. The granular ASBR consistently outperformed the non-granular ASBR in terms of COD removal efficiency. COD loading rates of 1.6 to 3.4 g/L/day and HRTs of 12 to 48 hrs were tested. The granular ASBR consistently removed 90% of the influent COD, whereas the non-granular ASBR showed unstable operation, with COD removals ranging from 30 to 90%.

Sung and Dague [1995] studied the effect of mixing and configuration on the performance of the ASBR operated at 35 °C and treating a non-fat dry milk substrate. They observed that intermittent mixing of the ASBR was as efficient as continuous mixing in terms of methane production and COD removal efficiency. An obvious implication of this finding is that significant energy savings can be obtained by mixing the ASBR only two or three minutes out of each hour of operation.

Reactor configuration was also seen to have an influence on the maximum MLSS values that could be obtained in Sung and Dague's study [1995]. Shorter, squatter reactors were able to accumulate more biomass than could be accumulated in the taller, thinner reactors. The squat ASBR (depth:diameter = 0.61) was able to achieve an MLSS level near 30,000 mg/L, whereas the tall ASBR (depth:diameter ratio = 5.6) could only achieve an MLSS level of about 20,000 mg/L under most loading conditions. An interesting observation was made after approximately 10 months of operation: the biomass in all of the ASBRs began to granulate. The tall reactors achieved higher degree of granulation than the squat

reactors, which was attributed to the greater depth to which the biomass had to settle in the tall ASBRs in order to remain within the reactor. That is, a greater depth of liquid (but equal volume) was decanted out of the tall ASBRs each cycle. Therefore, the poor settling particles were decanted out of the ASBR, thus selecting for the fastest settling biomass, which is a characteristics of granular biomass. The granulated biomass allowed for higher COD loads to be achieved than was possible previous to these experiments. COD loads up to 12 g/L/day at an HRT of 12 hrs was treated to 90% soluble COD removal efficiency.

A modification of the ASBR process is also studied at this time. The system consists of two ASBRs in series, with the first ASBR operating at thermophilic (55 °C) and the second at mesophilic(35 °C) temperatures. Another modification is biofilter at thermophilic (55 °C), followed by the ASBR at mesophilic temperature (Schmit, 1997). Both of the arrangement promised efficient performance, even with high organic loading.

D. High-Rate Anaerobic Treatment

1. Introduction

The anaerobic reactor which can retain high level of biomass population, and remove higher percentages of organic is known as the "high-rate anaerobic treatment." High-rate anaerobic reactor include the anaerobic contact process, the anaerobic filter, the upflow anaerobic sludge blanket reactor, the anaerobic expended bed reactor, the anaerobic fluidized bed reactor, anaerobic migration blank reactor (AMBR) and the anaerobic sequencing batch reactor (ASBR). Typical MLVSS concentration range from 30,000 to 50,000 mg/L for the UASB, 6,000 to 20, 000 for the anaerobic filter, and 10, 000 to 40, 000 mg/L for expanded/fluidized bed anaerobic reactors [Weiland and Rozzi, 1991].

According to Iza et al. [1991], the concept of high-rate anaerobic reactors is based on three fundamental aspects:

- "Accumulation, within the reactor, of biomass by means of settling, attachment to solids (fixed to mobile), or by recirculation. Such systems allow the retention of slowly growing microorganisms by ensuring that the mean solid retention time becomes much longer than the mean hydraulic retention time."
- "Improved contact between biomass and wastewater, overcoming problems of diffusion of substrates and products from bulk liquid to biofilms or granules."
- "Enhanced activity of the biomass, due to adaptation and growth."

Therefore, high-rate anaerobic reactors are able to decouple solids retention time (SRT) from hydraulic retention time (HRT) due to their ability to retain high population of microbial solids within the system. The various biomass retention technique such as attached growth or suspended growth, used by the different high-rate anaerobic reactors reported by HulshoffPol and Lettinga [1986]. The list is shown in Table 2-15.

2. Biomass Settleability and Granulation in High-Rate Systems

There are two general ways of microbial immobilization in high-rate anaerobic reactors. The first method is based on microbial sludge attachment to either stationary packing materials or particulate carrier materials. The second method is due to bacterial sludge aggregation to each other. The second method (Table 2-15) has received considerable attention in the literature due to high performance of some high-rate reactors which possess the ability to foster bacterial sludge aggregation.

The state of biomass in a biological reactor may be grouped into three classifications in relation to adhesion of microorganisms to each other. One of the three groups is the dispersed biomass which has little or no adhesion and therefore does not settle well. Because dispersed biomass does not settle well, the effluent from a biological reactor containing dispersed microorganisms is usually turbid due to suspended solids. Operations of a

biological reactor containing dispersed microorganisms usually results in a failure of the system due to biomass washout. The second group is the flocculent biomass which is a conglomeration of bacteria and other particles resulting in loose structure. Settling in a biological reactor containing flocculent biomass is characterized by the formation of a phase separation zone which moves downward as the biomass settles leaving clear effluent at the top. The third group is the granular biomass which is a conglomeration of bacteria resulting from adhesion of bacteria to one another as well as to organic and inorganic particles in the reactor forming a well defined structure. The structure of granular biomass is usually spherical in shape and is characterized by discrete and rapid settling. The development of granular biomass is important in anaerobic treatment because of rapid settling and high activity.

Table 2-15. Applied immobilization principles in high-rate anaerobic treatment system

Immobilized system	Treatment system
1. Bacterial attachment	
a) Stationary packing material (attached film)	<ul style="list-style-type: none"> •upflow anaerobic filter (AF) •downflow anaerobic filter (AFF)
b) Particulate carrier material (attached film)	<ul style="list-style-type: none"> •fluidized bed reactor (FB) •anaerobic expanded bed reactors (AEB) •floating bed systems •anaerobic gas lift reactor (AGLR)
2. Bacterial sludge aggregation reactors	<ul style="list-style-type: none"> •upflow anaerobic sludge blanket (UASB) •upflow anaerobic filter (AF) •anaerobic baffled reactor (ABF) •anaerobic migrating blanket reactor (AMBR) •anaerobic sequencing batch reactor (ASBR)

Hulshoffpol et al. [1986] did not include the ASBR and the AMBR system, which were developed in the Iowa State laboratory in the early and mid nineties respectively.

Recent granulation phenomenon and its importance in anaerobic treatment are attributed to research studies using the UASB reactor. Hulshoff Pol et al. [1983] summarized the factors affecting granulation in high-rate anaerobic reactors. The factors are

- Environmental conditions, such as temperature, pH, composition of wastewater, nutrients
- Type of seed biomass, i.e., with respect to settleability, specific activity, presence of inorganics
- The process condition conditions applied during start-up, such as loading rate and the amount of seed biomass.

Hulshoff Pol et al. [1983], in their research on granulation with the UASB, noted three phase in the granulation process in regards to system organic loading rate: no granulation was noted in Phase I which was defined as a system of organic loading rates of 2 g/L/d or less. Phase II was defined as a system having organic loading rates of 2-5 g/L/d. They defined this phase as the wash-out phase of lighter suspended solids which promotes a selection of the heavier fraction of the biomass in the reactor. Phase III, organic loading rate >5g/L/d is marked by the increase of granular growth.

According to Dolfin [1986], the mechanism of granulation is not completely known. however, the development of granulation shows that the high-rate reactor can select well settling sludges, thus allowing for easy system operation and high performance. Studies have shown that the granules in anaerobic reactors are composed of various species of methane forming bacteria. The species include Methanotrix-like organisms with wide variety of bacterial morphotypes. Others include Methanosarcina, Methanospirillum and methanotrix-Sohengenic [Dolfin, 1986; Hulshoff Pol et al., 1983].

E. High-rate Anaerobic Treatment of Dilute Wastewater at Low Temperatures

1. General

Anaerobic treatment has been revolutionized by the development of high-rate anaerobic reactor. Most of the problems encountered with the conventional continuous flow stirred tank reactor have been eliminated with the development of high-rate anaerobic reactors due to their high biomass holding capacity. McCarty [1968] presented the following points about high-rate anaerobic reactors:

- Treatment of relatively dilute soluble substrate is feasible
- Highly efficient treatment can be obtained at lower temperature
- The system is stable and readily responds to changes in organic or hydraulic loading, as well as to changes in temperature.

In addition, Hulshoff Pol et al. [1986] pointed out that the important features of high-rate anaerobic processes are:

- Anaerobic treatment becomes feasible at low ambient temperatures
- Very low strength wastes can be treated efficiently
- Compact installation can be applied
- Reactors are generally simple and low cost in construction
- Little operational and maintenance cost
- The process is applicable at very small and very large scales
- There is a high resistance to shock loads and environmental stress situations.

The features possessed by the high-rate systems have resulted in increasing application of anaerobic systems as efficient and energy producing process as well as energy and cost saving treatment systems. The treatment of low strength wastewater has been possible due to the development of high-rate anaerobic system.

Various descriptions have been documented to define the strength of wastewaters in relation to anaerobic treatment. Several papers, by Kato et al. [1994], Margaret et al. [1992],

Roockey and Foster et al. [1982]; Jewell et al. [1981], Switzenbaum and Jewell [1980], and Pretorius [1971,1972] define low strength or dilute wastewaters as those having COD values of 1000 mg/L or less. Low strength wastewater pose a special problem associated with the fact that at lower concentration of substrate, the diffusion rate (driving force) is low. The problem becomes more pronounced when biomass population is low, as in conventional continuous stirred tank anaerobic reactor. Therefore, an anaerobic process which can overcome these problems by fostering close proximity between biomass and substrate that is able to retain high populations of biomass and selects microbes which are able to grow at low substrate concentrations.

Anaerobic treatment of low strength wastewater is now feasible due to the development of high-rate reactors. Full scale applications are presently operating in tropical countries such as Brazil [Gomes et al. 1985, Vieira and Sonza. 1986], Columbia [Schellinkhout et al., 1992], and India [Draaijer et al., 1992]. Some of the full scale operation is going on in the Eastern Europe Countries such as Russia, Poland etc. (Personal Communication with Dr. Philip Heyden).

2. Previous Results with Dilute Wastewater under Psychrophilic Conditions

Past performance of anaerobic treatment of low strength wastewater in several popular high-rate anaerobic reactors under low temperature conditions will now briefly reviewed one by one.

a. Anaerobic contact process

i. Development. Fullen [1953] developed an anaerobic process for the treatment of packing house waste in the early 1950s. This process was later called " the anaerobic contact process" [Schroepfer et al., 1995; Schroepfer and Ziemke, 1959a, 1959b].

ii. Pilot plant studies. Schroepfer and Ziemke [1959a] reported on pilot plant applications of the anaerobic contact process for the treatment of various types of wastes

which included packing plant wastes, synthetic milk waste, fatty acid waste, wood fiber waste, and domestic sewage. At 35 °C, BOD₅ removal for synthetic milk ranged from 86% to 99.1% at influent BOD₅ ranging from 231 to 1354 mg/L, BOD₅ loading rate ranging from 0.77 to 4.42 g/L/day, HRT ranging from 7.2 to 7.35 hrs and sludge ages ranging from 8.85 to 3.76 days. At the same temperature of 35 °C, BOD₅ removal for domestic sewage ranged from 74.5% and 69.6% at influent BOD₅ of 243 mg/L and 221 mg/L respectively. At 25 °C, BOD₅ removal efficiency for milk waste ranged from 73.2% at influent BOD₅ of 646 mg/L, BOD₅ loading rate of 1.66 g/L/day, contact unit HRT of 9.34 hours, and a sludge age of 7.6 days to 100% at influent BOD₅ of 107 mg/L, BOD₅ loading rate of 0.31 g/L/day, contact unit HRT of 8.32 hours and sludge age of 24.7 days.

Simpson [1971] reported a successful pilot plant treatment of municipal liquid waste in the anaerobic contact process. The wastes consisted of 80% industrial origin and 20% domestic sewage. The waste strength ranged from 1100 to 1300 mg/L COD (300-400 mg/L BOD₅). The pilot plant consisted of a 3000 gallon storage tank, 370 gallon anaerobic contact tank followed by two 100 gallon (385 liters) settling tanks. The highest COD and BOD removal achieved was of 78% and 91% respectively, at 26.7 °C under a moderate loading of 0.037 lb BOD or 0.122 lb COD/ft³/day (0.59 g BOD/L/day).

iii. Full scale application. Steffen [1995] reported on the first full-scale anaerobic contact process for the treatment of meat packing wastes for the Wilson Meat Company at Albert Lea, Minnesota.

The second full scale application of the anaerobic contact process as a component of wastewater treatment plant was reported by Fall and Kraus [1961]. The plant was built at a suburban section of Peoria, Illinois. The plant was designed for 350 homes (1270) people. BOD₅ from the homes was averaged about 242 mg/L (525 mg/L COD). The suspended solids concentration was 368 mg/L TSS and 250 mg/L VSS. After 20 months of operation at a detention time of 22.4 hrs, data analysis showed that the anaerobic contact process

functioned best during the winter months (65 °F-71°F or 8.3 °C-15 °C) with a BOD removal of 42%. During the summer months (65 °F-71 °F or 18.3 °C-21.7 °C), the BOD removal averaged 14%. The cause of low efficiency of BOD removal during the summer months was said to be excess acids in the digester. Suspended solids removal were good at an average of 77% as compared to 60% obtained from Imhoff tanks, the alternate system at that time.

Kida et al. (1991) reported results of a study which compared efficiency of the anaerobic contact process, the upflow anaerobic sludge blanket (UASB), the anaerobic filter, and the anaerobic fluidized bed reactor (AFBR) for the treatment of low strength synthetic wastewater at 37 °C. They found that a sludge recycling process (that is the anaerobic contact process) was unsuitable for the treatment of wastewater having TOC concentration of less than 2000 mg/L. However, their study found that both the UASB and the AFBR were able to achieve TOC removal efficiency of 80% for the lowest-strength wastewater (TOC of 100 mg/L, which corresponds to a BOD of 250 mg/L). They also found that even at 15 °C, the AFBR was able to achieve 80% removals at the lowest substrate concentration and at HRT of three hours.

b. Anaerobic filter

i. Development. According to the Jewell [1987], the first anaerobic filter was developed in 1891 by Scott-Moneneff, and the first rock-filled upflow filter was initially tested in 1910. Coulter, Soneda and Ettinger [1956] also presented results of laboratory studies for the treatment of sewage in a rock-filled anaerobic filter.

The modern anaerobic filter was reported by Young and McCarty [1969]. They reported on a completely submerged, 12 liter laboratory-scale reactor which was filled with 1.0 to 1.5 inch quartzite stone (porosity of 0.42). The reactors were continuously feed at 25 °C. Their substrate consisted of a mixture of protein and carbohydrates, and a mixture of acetic and propionic acids. Waste strength ranged from 1,500 to 6,000 mg/L at hydraulic retention times (HRTs) of 4.5 to 72 hours, result in organic loadings of 0.43 to 3.40 g

COD/L/day. COD removal efficiency ranged from 93.4% at 72 hours HRT and 0.43 g COD/L/day loadings to a low of 36.7% at 4.5 hours HRT and 3.4 gCOD/L/day loading. The findings of Young and McCarty [1969] are as follows:

- The anaerobic filter is ideal for the treatment of soluble wastewaters
- Accumulation of biological solids in the anaerobic filter leads to long solids retention times (SRTs) and low effluent total suspended solids (TSS) and
- Because of the long SRTs, low strength wastes were successfully treated at a temperature of 25 °C.

ii. Laboratory studies. In addition to the initial studies by Young and McCarty [1969], Raman and Khan [1978] reported on laboratory scale anaerobic filter treatment of domestic sewage. Broken stones of 2 cm to 2.5 cm diameter were used as filter media which resulted in a reactor volume capable of treating 30 liters per day at five hours detention time. Settled sewage was used as a substrate at temperatures of 25 °C to 32 °C. Performance data showed BOD₅ removals ranging from 80 to 83% and suspended solids removals ranging from 80 to 86%.

Kobayashi, Sreustrom and Mah [1983] reported on the anaerobic treatment plant of domestic wastewater. Their reactor set-up utilized PVC packing material having a porosity of 97%. The packed column had a void volume of 4.4 gallons (16.7 liters). Treatment studies were done at temperature of 35 °C, 25 °C and 20 °C. Substrate was pumped into the reactor at a rate of 1 l ml/min which gave a packed contact bed time of one day. Influent COD concentrations ranged between 77 to 1170 mg/L (44-573 mg/L BOD), which resulted in organic loading rates of 0.048 to .54 gBOD/L/day (0.084-1.11 g COD/L/day). Twenty-nine percent COD removal (70% BOD removal) was observed at influent COD of 1170 mg/L (573 mg/L BOD). Gas production ranged from a low of 150 ml/day to over 1500 ml/day. The BOD₅ and COD removal and gas production were reported to be almost the same at 35 °C

and 25 °C. Based on average data, wastewater strength was at 288 mg/L resulting at organic loading rate of 0.288 g/L/day COD and average COD removed of 78%.

Viraraghavan et al. [1991] evaluated and compared the performances of three laboratory anaerobic filters treating septic tank effluent at temperatures of 5, 10 and 20 °C. They reported that the start-up of anaerobic filter at 20 °C is possible with no adverse effects on its future performance. The filter at 20 °C performed better than filters at 10 and 5 °C. The filter at 5 °C was the most effected by operational changes such as changes in HRT. The BOD values of the filter was generally higher than 30 mg/L, and the coliform levels were also high.

Viraraghavan et al. [1991] operated three anaerobic filters at 12.5, 21 and 30 °C treating dairy wastewater, with the HRT of 1-6 days. The COD loading was in the range of 0.63 to 4.03 g COD/L/day. The COD removal was 45% at temperature 12.5 °C and at HRT of 1 day with organic loading rate 4.03 g COD/L/day. Lin et al. [1982], Harrison et al. [1991] and Cordoba et al. [1988] also studied anaerobic filter treatment with different substrates, at low temperature conditions and at different HRTs. Their results significantly varied for different operating and environmental conditions.

iii. Pilot-plant studies. Raman and Khan [1978] reported on the treatment of sewage and septic tank effluent using three pilot plant anaerobic filters. One plant was located at NEERI campus at Nagpur, India, for raw sewage treatment. The other plants were located at a village near Singur, West Bengal, India to treat effluent from septic tanks.

A pilot-scale anaerobic filter treatment of low strength municipal wastewater was reported by Genung et al. [1978, 1982, 1985]. The 1985 paper was reported on a project sponsored by the U.S. Department of Energy. A 190 m³/day or 50200 gal/day reactor was located at Knoxville, Tennessee. The reactor dimensions were 4.9 m (16 ft) in diameter, and 5.4 m (18 ft) high with a 3m (10 ft) high packed section. A 3 m section was packed with 3-in polypropylene pall rings. About 7.5 m³ was allowed for head space above the normal liquid

volume. The plant was operated for 800 days during August 1981 to October 1983. Temperature of the incoming sewage could be raised using a feed preheater up to 25 °C, however, the monthly averages of feed wastewater ranged from a low of 12 °C to a high of 27 °C. Feed flow rates into the reactor were gradually increased until the nominal design level of 135 L/min (50,000 gal/day) was reached. Pollution loading rates ranged from 0.1 to 0.7 g/L/day TSS and from 0.1 to 0.4 g/day BOD. TSS removal efficiencies were 78 to 93% at loading rates in the range of 0.12 to 0.73 g/L/day and was found to be insensitive to temperature change. Removal efficiencies of 63 to 70% were observed for BOD at loading rates of 0.13 to 0.40 g/L/day and 50 to 70% COD removal at loading rates of 0.35 to 1.2 g/L/day. Based on the result of the pilot plant studies, it was recommended that the system should be attractive to small communities for treatment of low-volume sewage flow of about 50,000 to 750,000 gpd.

Other pilot plant studies on the use of the anaerobic filters for sewage treatment were done in the state of Parana, Brazil [Gomes. 1985]. The studies started in 1980 and involved: use of anaerobic filter for the treatment of septic tank effluents; use of imhoff's type tanks followed by the UASB, and use of UASB reactor with just 2 to 3 hours HRT for primary treatment. The pilot plants which utilized anaerobic filters for the treatment of septic tank effluents showed about 80% BOD₅ removal but the anaerobic filter pilot plants started clogging the media and their operation (which would involve occasional cleaning of the media) was found to be too expensive, so the anaerobic filters were abandoned.

c. UASB reactor

i. Development. According to the Jewell [1987], the first upflow anaerobic sludge blanket (UASB), the biolytic tank, was used by Winslow and Phelps in 1910. The anaerobic sludge blanket reactor was used again in the 1950's [Coulter, Soneda and Ettinger, 1956 and 1957]. Modern application of the UASB reactor is attributed to the 1970s studies by Lettinga and co-workers in the Netherlands [Jewell, 1987]. A report on the UASB was

published by Lettinga and co-workers in the Netherlands [Jewell, 1987]. A report on the UASB was published by Lettinga, van Velsen, Hobma, Zeeuw and Klapwijk [1980], which showed treatment results of some laboratory and pilot plant studies that started in 1971. These studies utilized various types of wastewaters having influent COD concentration ranging from 1500 to 10,000 mg/L, organic loading rates ranging from 4 to 14 g/L/day and HRTs of 5 to 48 hours. Results showed COD removals ranging from 65 to 97% at various temperatures.

ii. Laboratory studies. A laboratory study of the UASB treatment of municipal sewage was conducted in Spain by Sanz and Fdz-Polanco [1989]. The laboratory study made use of a 5.2 cm diameter reactor having a total volume of 1.42 liters. Domestic sewage having a COD of 150 to 590 mg/L, BOD₅ of 325 mg/L, and TSS of 190 mg/L was used as substrate. Studies were conducted at room temperature with fluctuations ranging from a 15 °C to 25 °C. Temperatures as low as 9 °C were occasionally recorded. Organic loading rate ranged from 0.5 to 4.5 g COD/L/day. Hydraulic retention times ranged from 2.6 to 7.2 hours, based on an active reactor volume of 0.40 liters. Results of the study showed occasional drops in percent COD removal at higher organic loadings with COD removal ranging from about 50 to 90%.

Grant et al. [1995] studied the effects of temperature and organic loading on the performance of UASB reactors of volume 3.2 L. feeding a synthetic substrate consisting of beef consomme', and micro and micro-nutrient compounds. Temperature range was 10-42 °C, and organic loading was 2-3 g COD/L/day. The COD removals at 10 °C for organic loading rate of 2-10 g COD/L/day was in the range of 88 to 55%. However, for the temperature at 42 °C and organic load of 10 g COD/L/day, the COD removal was about 88%. They also determined the kinetics value of K_s and K, by assuming that UASB reactor kinetics follow the Monod and Arrhenius equations in the temperature range of 10-42 °C.

iii. Pilot plant studies. Lettinga et al. [1981, 1983] reported results of a 120 liter pilot plant UASB reactor treatment of domestic sewage. The reactor was seeded with 75 liters of granular type anaerobic sludge which was cultivated on a sugar beet wastewater. The reactor was kept at 15 °C [Lettinga et al., 1981]. Results obtained during the first year of study showed 65 to 85% COD reduction (based on filtered effluent and unfiltered influent) at 48 to 8 hours HRT and at ambient temperature of the sewage (7 to 12 °C at winter time and 20 °C at summer time condition). During severe rainfall and the consequent high dilution of the sewage, COD removal was between 50 to 75%. Results also showed that 65 to 75% of the COD removed was converted to methane. Gas production was sensitive to temperature changes. For example, average gas production was 220 L/kg COD during summer compared to 135 L/kg COD during winter.

Another UASB pilot plant treatment of sewage using a 6000 liters reactor was also reported by Lettinga et al. [1983]. The study started from October 1979 to March 1980. The seed biomass for this pilot plant was non-granular sludge. The study was conducted at 20 °C to evaluate the use of UASB reactor for sewage treatment under tropical conditions. During dry weather conditions, COD removal ranged from 55 to 75% but fell to about 30% during rainy weather conditions. The reactor biomass was changed in 1981 to granular sludge which resulted in additional COD removal of 38 to 40% at both dry and rainy weather conditions in comparison to the results obtained from non-granular seed.

Results of the 6,000 liters pilot plant reactor for 1982 to 1983 operation was reported by Grin, Roersma and Lettinga [1983a, 1983b, 1985]. COD and TSS of the raw sewage ranged between 100 to 900 mg/L and 10 to 700 mg/L, respectively. Treatment was done at 8 hrs HRT and at temperatures of 19 to 15 °C, 12 to 11 °C, and 10 to 9.5 °C. Between 20 °C and 14 °C, the COD removal was hardly influenced by the decrease in temperature. Between 14 °C and 10 °C, the soluble COD removal efficiency was slightly lower than observed at above 14 °C. It was observed that at a temperature below 10 °C the amount of suspended

solids in the effluent exceeded 200 mg/L at influent COD of 500-600 mg/L. With COD removal of 60 to 80%, the UASB study was said to be satisfactory but further treatment, such as aerobic post-treatment, for meeting COD discharge requirement and for the removal of nitrogenous compound was recommended [Lettinga et al., 1983].

Kiriyama et al. [1992] reported on a 76,700 liters pilot plant UASB reactor in Japan which was operated at a HRT of 1.8 hours. The reactor was seeded with 30,400 liters of granular biomass which has BOD concentration ranging from 154 to 166 mg/L. The reactor start-up was at 35 °C, but the reported data was obtained at temperatures of 27.5 °C, 23.9 °C and 12.4 °C. Temperature changes were accomplished by a drop of 2 °C every four days. The reported BOD₅ removal was 73.3% at 27.5 °C, 79.7% at 23.9 °C and 70.5% at 12.4 °C.

Van der Last and Lettinga [1992] reported on pilot plant treatment of domestic sewage using the UASB reactor which were operated under expanded bed and fluidized bed conditions. The experiments were resulted by poor performance of up-scaled (6,000 and 20,000 liters) UASB sewage treatment using granular biomass at temperature below 10 °C. The poor performance was attributed to poor sludge contact and decreased hydrolysis and removal of suspended solids.

Singh et al. [1996] conducted feasibility study of anaerobic treatment of low strength synthetic wastewater (500mg-COD/L) using UASB reactor at ambient temperature conditions (20-35 °C). The HRT was 3 hr. corresponding to organic loading of 4 g-COD/L/day. 90 to 92% COD and 94-96% BOD was achieved at those temperature.

Rebac et al. [1995] reported on expended granular sludge blanket reactor (EGSB) study, treating a mixture of VFA under psychrophilic conditions (10-12 °C). The experiment were resulted 90% COD removal with 500-800 mg/L influent substrate concentration. The reactor operated with HRT between 2.5 to 1.6 hr, and organic loading rate upto 12 g-COD/L/day.

The expanded UASB study was conducted using a 100 liter pilot scale reactor having superficial liquid velocity in the range of 4 to 8 m/hr. The reactor was seeded with 54 liters of granular biomass. The sewage treated was presettled domestic sewage whose temperature ranged from 15 to 20 °C in the summer and 6 to 9 °C in the winter. Total COD of the settled sewage ranged from 120 to 391 mg/L with soluble COD ranging from 77 to 291 mg/L. During dry weather conditions, COD removal ranged from 31% to 80% and between 18% to 41% at wet weather conditions.

The fluidized UASB study was conducted in a 205 liter pilot scale reactor using a superficial liquid velocity of 24 m/h at the same temperature range and sewage strength as in the expanded bed UASB. It was concluded that it did not offer any prospect for sewage treatment because of excessive volatile acids in the reactor which made the fluidized UASB act as a pre-acidification reactor [Van der Last and Lettinga, 1992].

Kato et al. [1994] studied the performance of UASB reactor with low strength soluble wastewaters, containing ethanol or whey, at 30 °C. The treatment of ethanol containing wastewater resulted 95% COD removal at organic loading of 0.3 to 6.8 g COD/L/day with influent concentrations of 422-943 mg/L. They also observed that lower the COD concentration decrease the removal efficiency, with a sharper decrease below 200 mg/L.

iv. Full scale application. The most extensive application of anaerobic processes for low strength waste treatment came from numerous pilot studies in Brazil [Gomes, 1985; Souza, 1986; Vieira and Souza, 1986; Vieira and Garcia, 1992]. The studies started in the early 1980's and initially made use of both UASB and the anaerobic filter. The anaerobic filter was abandoned in the early 1980's due to clogging problems. By 1985, there were about 20 anaerobic pilot plant for sewage treatment in Brazil [Gomes, 1985]. The first full scale UASB plant for sewage treatment in Brazil was reported by Viera and Souza [1986]. The performance of the full scale plant was again reported by Viera and Garcia [1992]. Removal efficiency during the years of operation ranged from 54% to 65% for total

COD, 74% to 84% for soluble COD, 60% to 72% for BOD, 62% to 76% for TSS and a methane yield of 0.09 to 0.15 m³/kg COD. The system is considered to be a success. No heating is required at the ambient temperature of 15 °C and above. However, the effluent is discharged into a stabilization pond for polishing.

Draaijer, Maas, Schaapman and Khan [1992] reported on a 1,200,000 liters UASB reactor for sewage treatment. The full scale plant which has been in operation since April 1989 is located at Kanpur, India. Sewage to the plant has an average COD of 563 mg/L, a BOD of 214 mg/L and a TSS of 418 mg/L. At the system HRT of 6 hours, the average reduction in COD, BOD, and TSS are 74, 75 and 75% with biogas yield of .05 to 0.10 m³/kg COD. Summarizing the performance of the plant, the state that the plant has constant efficiency even at low ambient temperature of 20 °C except for total gas production. Effluent from the plant is discharged into a pond for polishing. Due to the good performance of the plant, the authors also reported that a 3,360,000 liters full scale is being constructed at Mirzapur, India which has post-treatment system consisting of a pond with one day retention time.

Schelinkhout and Collazos [1992] reported on a full scale UASB plant for sewage treatment in Columbia. The 8 MGD plant was completed in September of 1990 and was constructed after a four year (1983-1987) pilot plant study. The plant is designed for an average HRT of 5.2 hour with a facultative lagoon for effluent polishing. The actual performance of the system is not yet available but the start-up performance indicates that the UASB will achieve an 85% BOD removal.

d. Anaerobic expanded bed reactor

i. Development. Upon the introduction of the modern anaerobic filter by Young and McCarty [1969], there was interest in the 1970s in developing attached biomass reactors that will be able to handle fine suspended solids without clogging. As a result of this

need, the anaerobic attached-film expanded bed reactor (AAFEB) for waste treatment was developed in 1981 by Jewell [1987].

ii. Laboratory studies. Switzerbaum and Jewell [1980] reported on a laboratory expanded bed reactor for treatment of low strength synthetic wastewater. Three laboratory reactors were used, each having a 1 liter of total volume. One hundred and sixty grams of support media composed of aluminum oxide having approximate diameter of 500 micro meter and bulk density of 0.60 g/cm was used, which was estimated to displace a liquid volume of 90 ml. Hydraulic retention time varied from 6 to 0.33 hours at organic loading rates of 0.8 to 4.8 g COD/L/day and treatment temperatures of 10 °C, 20 °C and 30 °C. During operation the bed expanded from 400 ml to 500 ml by means of effluent recycling. Synthetic substrate was used in the study and comprised of glucose, yeast extract, ammonium chloride, monobasic potassium phosphate, dibasic potassium phosphate, and sodium bicarbonate with respective concentration of 29.9, 0.1, 7.5, 2.5, 1.0 and 55.0 g/L. The substrate was then diluted to give concentration ranging from 50 to 600 mg/L COD. The COD removal efficiencies obtained for six different HRTs ranging from 20 minutes to 6 hours were at a low of 38% to over 90% at the various temperatures.

Jewell, Switzenbaum and Morris [1981] reported on the anaerobic expanded bed reactor treatment of primary settled domestic wastewater using a one liter laboratory reactor. Media of biomass attachment was polyvinyl chloride particles having a diameter of 1 mm or less. System HRT ranged from 5 to 180 min at a treatment temperature of 20 °C. Primary settled domestic sewage was used as substrate. The primary settled sewage had a minimum COD of 88 mg/L, maximum COD of 206 mg/L, and an average COD of 186 mg/L. Maximum TSS was 186 mg/L, with 40 mg/L minimum TSS and average TSS of 86 mg/L. Organic loading rates ranged from 0.65 to 3.0 g/L/day. The studies lasted for about 200 days. Results showed good TSS removal with effluent solids ranging from 3 to 20 mg/L. COD

removal was higher at about 80% for 2 to 5 hours HRT. Based on the result, they concluded that the system was a viable alternative for the treatment of low strength wastewaters.

iii. Pilot plant studies. Three anaerobic attached-film expanded bed reactor (AAFEB) pilot plant for sewage treatment were built in the early 1980s by the EPA [Brown et al., 1985]. In the pilot scale studies, maximum total COD removal obtained was 75%. A major problem with the pilot plant studies was related to sulfate reduction which limited methane production. When methane production occurred, the methane bubbles were said to be attached to and float with the sand media. However, it was concluded that as the design of the reactor improves, the AAFEB may prove to be a viable alternative for treating municipal sewage.

e. Anaerobic fluidized bed

i. Development. According to Stathis [1980], the early work on anaerobic fluidized bed reactor (AFBR) were performed by Beer in 1970. By 1983, numerous types of wastewater were treated using the anaerobic fluidized bed [Henze and Harreemoes, 1983].

ii. Laboratory studies. Sanz and Fdz-Polanco [1986] reported on the laboratory treatment of domestic sewage using a 0.54 liter anaerobic fluidized bed reactor (AFBR). Treatment temperature of 10 °C to 25 °C were used. Active volume of the reactor was 0.23 liters after the addition of 0.14-0.28 mm diameter support material whose density was 1.94 g/cm³ and a porosity of 57%. Volumetric expansion of the system was 20% at a fluid velocity of 0.11 cm/sec. The reactor was run for a duration of 320 days treating domestic sewage having a COD value of 500 mg/L (BOD₅ of 325 mg/L) and a TSS value of 190 mg/L. COD removal was between 60 and 90% at COD loadings of less than 1 g/L/day and about 60% at loading rates of 3 to 5 g/L/day.

Maragno and Campos [1992] reported on a laboratory treatment of synthetic wastewater with a mean COD of 557 and 700 mg/L in a 10.5 liter AFBR. The synthetic

wastewater was composed of bovine liver extract, glucose, sodium bicarbonate, and ammonium acetate. The reactor was seeded with sand that was retained between sieves with openings of 0.21 mm and 0.149 mm, as carrier material and operated at ambient temperature of 13 °C to 31 °C and at two stages of HRTs of 1.0 hour and 1.5 hours. A mean COD removal of 62% was achieved at a 1.0 hour HRT for the first phase and 71% removal at a 1.5 hour HRT for the second stage.

iii. Pilot plant studies. Jeirs, ehlers and Witkowski [1985] summarized results of two pilot plants for AFBR treatment of sewage in New York. One plant used sand and the second utilized GAC as carrier materials. The maximum BOD removal for GAC reactor was 78%.

f. Anaerobic sequencing batch reactor (ASBR)

i. Development. The development and operating principle of ASBR was described initially in this chapter. Several laboratory studies with the ASBR already conducted with different operating and environmental conditions, along with various industrial and agricultural wastes. One ASBR pilot plant was operated at Cedar Rapids, Iowa treating wastewater from a starch manufacturing company. A full-scale ASBR under construction at Wellington, Colorado, for the treatment of swine waste. Another full scale ASBR is under construction at Tama, Iowa for the treatment of Excel waste.

ii. Laboratory studies. Piddaparti [1991] and Schmidt [1992] studied the treatment of swine waste using the ASBR at different temperatures of 35, 25 and 20 °C. Piddaparti found equivalent COD removal and VSS destruction at 35 °C and 25 °C. The equivalent treatment performance at 35 °C and 25 °C was explained by a logic that more solids were built up at 25 °C due to the low endogenous decay rate which compensates for

decrease in reaction rate from 35 °C to 25 °C. Schmit found at 20 °C that the ASBR process was capable of sustaining volatile solids destruction (40 to 60%) over a variety of loadings.

Ndon et al. [1994] carried out a detail ASBR study for treating dilute synthetic wastewater at temperatures 35, 25, 20 and 15 °C. He operated four ASBR reactors with HRTs of 48, 24, 16 and 12 hrs and concentration of 1000, 800, 600, 400 mg/L at all temperatures. The ASBRs were seeded with primary anaerobic digester sludge obtained from the Water Pollution Control Plant at Ames, Iowa. He observed that soluble COD removals at the 48 and 24 hr HRT were in excess of 90% at all feed concentrations and temperatures. At the lower HRTs of 16 and 12 hr, the soluble COD removals were in excess of 85% at 35, 25, 20 °C, except for 15 °C. He observed in excess of 85% SCOD removal for the lower COD concentration of 600 and 400 mg/L at 15 °C. The reasons he mentioned for not performing well of concentrations 1000 and 800 mg/L at 15 °C was due to the excessive loss of solids in the effluent. He found methane production was very close to the theoretical value of 0.35 liters per gram of COD.

From the literature of this chapter, it is evident that no formal study has been conducted on the ASBR treatment of low strength wastewater at psychrophilic temperatures as below as 5 °C. The main purpose of this research presented in this document was to applicability of the ASBR process for the treatment of dilute wastewater at various psychrophilic temperatures ranging from 25 to 5 °C and at various HRT of 24, 16, 12, 8 and 6 hrs.

CHAPTER 3

EXPERIMENTAL SETUP

Three identical reactor systems, each consisting of one ASBR, a substrate feed system, an effluent decant system, a gas mixing system, and a gas measuring system, were used for the experiments in this research. The ASBRs were constructed in the Engineering Research Institute (ERI) Machine Shop at Iowa State University. Excessory materials, such as tubing, clamps, fittings, and connections, were purchased at the central stores and chemistry store of Iowa State University.

A. Reactor Configuration

1. Reactors

The body of the reactors were constructed using 12 mm (0.5 in) thick Plexiglas and were cylindrical in shape. The reactors were 24 inches in height and 4.5 in internal diameter. The total volume of each of the reactors was 6.25 liters of which 6 liters was used as working (fluid) volume and the remaining 0.25 liters for head space. The reactors were identical in all respect. The dimensions and descriptive schematic of one of the reactors are shown in Figures 3-1 and 3-2.

Six sampling ports were installed at one liter volume intervals from the bottom of the reactor. One additional sampling ports were installed at 4.5 liter volume from the bottom of the reactor. The sampling ports were 6 inches long with 0.5 inches inside diameter and were made of stainless steel tubes. Compression fittings with one inch by one inch by 0.25 inch Plexiglas reinforcement anchors were used to reinforce the sampling ports.

The top and bottom of the reactors were flanged with plate of having the same outside diameter of 8 in. The plates and flanges were 0.5 inches thick. Both the plates and the flanges of each reactor (top and bottom) had twelve, 3/8 inch holes in which threaded rods were used to fasten the reactor into a single unit. The threaded rods were tightened with 0.625 inch nuts.

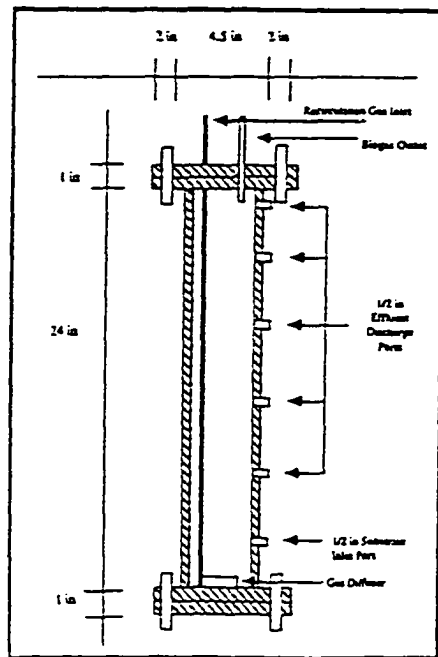


Figure 3-1. Dimensional view of one of the three identical ASBRs used in this study

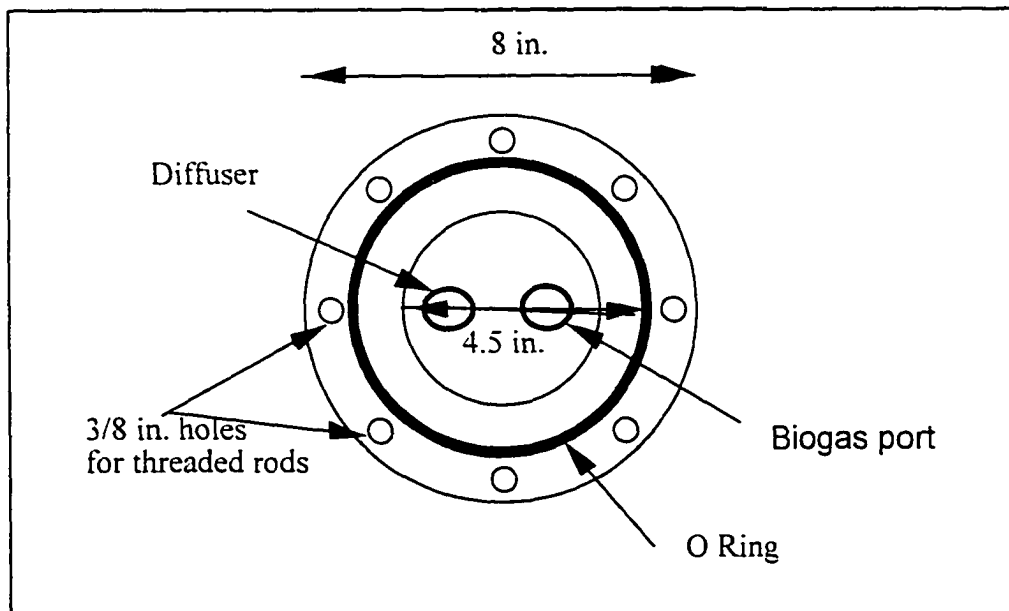


Figure 3-2. Schematic of a sectional view of the top of ASBR

The top plate of each ASBR contained two holes. One of the holes was used for biogas outlet. The other one was fitted with a 0.5 inch inside diameter stainless steel tubing 27 inches long, secured with a compression fittings on the reactor lid. The stainless steel tubing extended to the bottom of the reactor where it was welded to a circular stainless steel ring having holes for gas delivery for mixing. A Schematic of a sectional view of one of the reactors is shown in Figure 3-2.

The volumes were calibrated after reactor construction, using measured amount of tap water. The reactor were connected using tygon tubing and connectors. Peristaltic pumps were used for the feeding of the substrate, effluent withdrawal and biogas recirculation for mixing.

Wood shelving was constructed for organized placement of pumps and timers. A total of nine pumps were used. Five pumps were 60 rpm constant speed drives (Cat. No. L-07543-30, Masterflex, Cole-Parmer Company), while one pumps was 30 rpm constant speed drive (Cat. No. L-07543-60). The three gas recirculation pumps selected were 6-600 rpm variable speed drive (Cat. No. L-07553-50). All the pump drives were fitted with standard size 18 pump heads (Cat. No. L-07018-20). Three automatic programmable timers (Cat. No. L-01750-5) were used for each ASBR, one for the influent pump, the second for the effluent pump, and the third for the gas recirculation system. Masterflex (Tygon) 6 size was used for all pump heads. The connections between the pump tubing and the inlet and the outlet tubing were two way, 1/4 to 5/8-in polyethylene connectors (Chemistry Stores, ISU). Figure 3-3. shows the experimental set-up of the ASBRs system used in this research.

2. Gas/Foam Separation System

The gas and any liquid vapor/foam exited the reactor through one of the top holes on the plate of the reactor and was connected by an tygon tubing to an aspirator bottle for gas-liquid separation by way of tygon tubing. The gas-liquid separation was needed to prevent liquid from entering the gas line and clogging the tubing. The gas-liquid separation was

needed to prevent liquid from entering the gas line and clogging the tubings. The 4 liter aspirator bottles, were used for the gas-liquid separation, and had a discharge port located in its side, near the bottom. This port was occasionally opened to drain any accumulated liquid in the bottle. The top of the bottle was closed with a number 10 rubber stopper and sealed with silicon sealant to prevent any gas leaks. Three holes were drilled in the rubber stopper. Each hole was fitted with a piece of glass tube. The sides were sealed with silicon to prevent leaks. One of the three glass tubes extended to about one inch from the bottom of the aspirator bottle and was used to deliver the gas and accompanying liquid vapor from the reactor. The other two glass tubing were short and were inserted just to the inside end of the rubber stopper. One of the short glass tubes was used to deliver gas to the gas recirculation pump and back into the reactor for mixing through the second hole on the top plate of the reactor.

3. Biogas Recirculation System

Mixing was accompanied by recirculating the biogas in a closed loop to the bottom of the ASBR through a stainless steel ring diffuser system. The diffuser system consisted of a 5/8-in diameter pipe which extended from the bottom of the ASBR through the top plate of the ASBR. The pipe was positioned along the edge of the ASBR so that at the bottom of the pipe a 1/4-in diffuser ring could be attached (Figure 3-3). The diffuser consisted of two concentric ring connected by three stainless steel tubes. The inner and outer rings were 3 1/2-in and 2 1/2-in diameter, respectively. The inner ring had six 1/32-in equally spaced holes and outer ring had eight 1/32-in equally spaced holes drilled in the bottom to provide the outlet of the diffuser for mixing. All parts of the diffuser system were hollow to allow the recirculated biogas to flow them.

The recirculation system worked as follows: Biogas produced from methanogenic activity exited the ASBR through a port described earlier and was discharged to the aspirator bottle. When the ASBR was mixed, a peristaltic pump turned on and pulled biogas

out of the aspirator bottle via one of the top ports. The discharge end of the pump was connected with 5/8-in Nalgene tubing to the stainless tube diffuser pipe. The biogas discharged through the ring diffusers and rose to the surface of the ASBR, thereby mixing the system and completing the biogas loop.

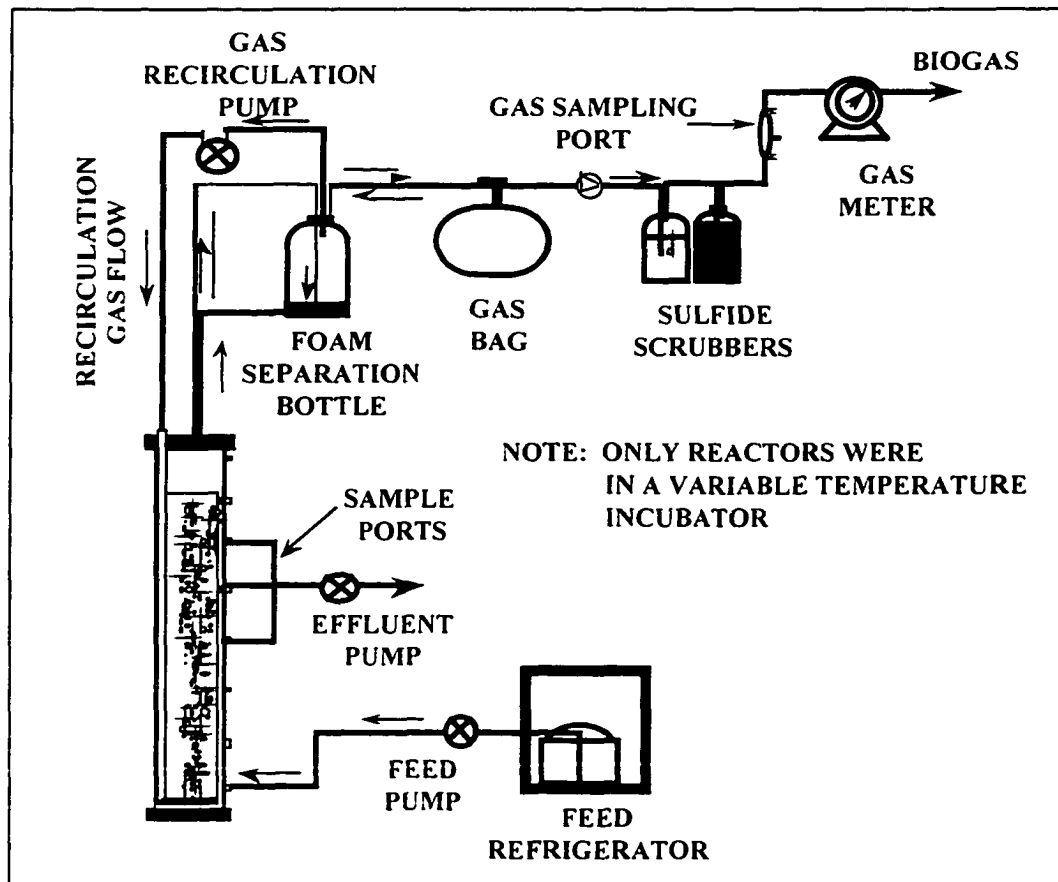


Figure 3-3. Experimental Set-up of ASBR

4. Biogas Collection and Measurement System

The biogas that was produced ultimately left the aspirator bottle (gas/liquid separation bottle) via the gas exit port to the gas measurement system (Figure 3-3). The gas exit port was connected to a water observation bottle (one liter volume) with 3/8-in Nalgene tubing. The observation bottle had a rubber stopper with inlet and outlet glass tubes extending out the top and bottom of the stopper. The inlet glass tube extended 1/2-in below the water level in

the bottle so that the bubbles of gas could be visually observed. There were two valves between the aspirator bottle and the observation bottle. The first valve was a three-way T-connection that was connected with the tubing to the aspirator bottle, the observation bottle, and to a gas bag which had a volume about 4 liters. The bag served as a reservoir of gas and provided gas to the ASBR by deflating during decanting to avoid drawing a vacuum on the system. Just before the observation bottle there was a one way check valve to prevent gas being drawn back through the observation bottle and downstream equipment during decanting.

A hydrogen sulfide scrubbing bottle (one liter volume) containing steel wool pads was placed after the observation bottle. The inlet and outlet of the scrubbing bottle were identical to the observation bottle, with the exception that there was no water in the scrubber. Hydrogen sulfide produced in the ASBR was removed from the gas stream by reaction with ferric oxide to form ferrous sulfide, which form a black precipitate in the scrubber bottle.

After the scrubber bottle, the gas passed through the cylindrical blown-glass gas sampling compartments (4-in long and 1-in diameter) which had a 1/4-in port with a rubber septum placed snugly into it (Figure 3-3). This compartment was used to sample the biogas with a syringe for gas composition analyses using chromatography.

The final stage of the biogas collection system was the gas meter. After exiting the gas sampling compartment, the biogas entered a gas meter, which measured the volume of biogas produced over time. The gas was vented to the building air ventilation system. All tubing used in the gas collection and measurement system Nalgene brand tubing with an inside diameter of 3/8-in and a wall thickness of 1/16-in. Appropriate connectors were placed between all parts of the system so that any one component could be taken out and repaired without disrupting the flow of biogas.

5. Substrate Feed and Effluent Decant Systems

The substrate used in the experiments was prepared daily (described in the Experimental Procedure Section) in 40-liter polyethylene carboys. A 6-in section of 3/8-in glass tubing was connected with 3/8-in Nalgene tubing to the substrate feed pump. The glass end was inserted into the carboy while the other end of the tubing was connected to the pump head tubing in the identical fashion, as earlier described. The pumps were 60-rpm constant speed peristaltic pumps and were connected to a timer which turned them on and off at the appropriate time. The discharge end of the pump was connected with 3/8-in Nalgene tubing to the bottom side port of the ASBR.

The decanting pumps, timer, and tubing were identical to those used for the substrate feed system. The selected side port for decanting was connected to the decant pump, which discharged the effluent to a sanitary sewer drain.

B. Substrate Feed Preparation

The substrate used in this study was a synthetic waste which consisted of non-fat dry milk (NFDM), sodium bicarbonate alkalinity, and trace mineral solution. The synthetic substrate (NFDM) was used as because it is stable, relatively inexpensive, and almost completely soluble. It contains the necessary nutrients, can be stored for a long time and can be used to prepare desired substrate strengths. NFDM was also used in previous ASBR studies in Iowa State University [Habben, 1991; Kaiser, 1991; Sung, 1995]. Properties of the NFDM are shown in Table 3-1.

Sodium bicarbonate (NaHCO_3) was used to maintain suitable buffering. The bicarbonate alkalinity was added at 1.0 g NaHCO_3 , as CaCO_3 , per 1.0 g COD. Therefore, the alkalinity added was in increasing proportion with increasing COD concentration in the substrate. Five trace minerals (Fe, Zn, Ni, Co, and Mo) were added to the substrate for supplying adequate nutrients. 0.5 ml of trace minerals were added for each g of NFDM. The composition of the trace mineral solution is shown in Table 3-2.

The synthetic substrate was prepared each day. But the quantity was different in different time depending upon the loading conditions. For example, for the preparation of NFDM synthetic substrate of COD concentration 600 mg/L and 30 L of substrate volume, 17.31 g NFDM, 17.3 g of NaHCO_3 and 15 ml of trace minerals were added. The measured NFDM, NaHCO_3 , and trace mineral solution were first mixed with water using a blender for about 20 seconds to obtain a well dissolved and homogenous substrate. The paste was then

Table 3-1. Properties of the non-fat dry milk (NFDM)

Parameter	Value
Chemical Oxygen Demand (COD), g-COD/g-NFDM	1.04
Five-day Biochemical Oxygen Demand, g-BOD ₅ /g-NFDM	0.47
Total Kjeldahl Nitrogen (TKN), g-TKN/100g-NFDM	5.40
Total Phosphate, g-PO ₄ /100g-NFDM	2.20
Lactose, g/100g-NFDM	51.00
Protein, g/100g-NFDM	>36.00
Fat, g/100g-NFDM	<1.00
Ash, g/100g-NFDM	8.20
Trace Minerals ^a	
Iron, mg/kg of NFDM	4.60
Nickel, mg/kg of NFDM	1.00
Cobalt, mg/kg of NFDM	0.80
Molybdenum, mg/kg of NFDM	3.00
Zinc, mg/kg of NFDM	15.00

^a Source of data is Swiss Valley Farms Inc., Davenport, Iowa

poured, and the mixing container rinsed, into the carboys. The volume of the substrate was then brought to desired amount by adding tap water, depending on the HRT of the system. The volume of feed substrate prepared was 2 liters in excess of the amount fed to the ASBRs on a daily basis. This was done to ensure that air would not be pumped into the reactor, which would occur if the carboys were completely empty. The remaining two liters were poured down the drain prior making the new feeds, and the carboy was cleaned and rinsed to avoid bacteria.

Table 3-2. Recipe for trace minerals stock solution

Chemical Compound	Concentration	Criteria (1)
$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	36.60 g/L	Fe = 200 ppm
ZnCl_2	2.08 g/L	Zn = 20 ppm
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	4.05 g/L	Ni = 20 ppm
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	4.04 g/L	Co = 20 ppm
$\text{MoCl}_2 \cdot 4\text{H}_2\text{O}$	3.61 g/L	Mn = 20 ppm

(1) Fe = 200 ppm means that 200 ppm of iron were added per million parts of NFDM. The numbers shown are the ratios of the element to the quantity of NFDM on a dry weight basis.

Tap water was used in the substrate preparation in place of distilled or deionized water for two reasons. First, the inadequacy of the logistic system in the laboratory, required relatively long time to obtained sufficient distilled water to make all of the substrate feeds. Second, the tap water provided other essential nutrients for the biological systems, most notably, calcium, magnesium, and sulfur (sulfate). The average composition of the Ames' tap water is shown in Table 3-3.

C. Biological Seeding of the ASBR

Anaerobic biological seed/granules was obtained from the anaerobic digester at the city of CedarRapids Water Pollution Control Plant. The ASBRs were purged with methane, after which the seed was pumped into the sealed ASBRs. Approximately 2-2.5 liters of the seed were pumped into each reactor, so that it makes the MLVSS concentrations about 10,000-12,000 mg/L. The liquid volume of the ASBR was adjusted to 6 liters with tap water. During and after seeding, the ASBRs were purged with methane to remove all of the oxygen from the system. Gas chromatography was also used to determine the approximate O₂ composition of the gas in the reactor. Methane purging was continued until the oxygen peak was diminished to below detectable levels (O₂ < 1% of gas). The ASBRs were then fed with 1 liter of the respective substrate solutions and the gas meter readings were recorded. At this point, the experiment was considered started.

Table 3-3. Composition of Ames municipal water.

Component	Average Concentration, mg/L
Calcium, as Ca	130-140
Magnesium, as Mg	20- 30
Sulfate, as SO ₄ ²⁻	80-120
Iron, as Fe	0.09- 0.11
Carbonate hardness, as CaCO ₃	40- 60
Noncarbonate hardness, as CaCO ₃	120-130
Alkalinity, as CaCO ₃	40- 60
pH	6.8-7.2

D. ASBR Start-up

Each of the ASBRs was operated independently during the experiment except the feed was supplied from one carboys. All the reactors were started at operating temperature 25 °C with a hydraulic retention time (HRT) of 24 hours. During the start-up, the ASBRs were operated with a cycle length of 6 hours, corresponding to four cycles per day. The substrate concentration was 1000 mg/L for all the reactors. During this time, all the leaks were detected and fixed. After operation of 65 days, the reactors came in equilibrium conditions. During the equilibrium conditions, the organic removal performance of the reactor were highest, and the removals were closely matched with the methane production. In this time, the granules settled well and clear supernatant was apparent. After proper start-up of reactors in this conditions, the reactors temperatures and HRTs were changed according to proposed plan.

E. Reactor Operation

The synthetic milk substrate of COD concentration 600 mg/L was used for 24, 16, 12, 8 and 6 hours HRT at temperatures 25, 20, 17.5, 15, 12.5, 10, 7.5 and 5 °C. Two HRT were used for each temperature condition. The reactor 1 was operated at 24 and 12 hour HRT. reactor 2 was operated at 16 and 8 hour HRT and reactor 3 was operated at 12 and 6 hour HRT at all temperatures. After collecting data for 24, 16 and 12 hours HRT for reactors 1, 2 and 3 at each temperature, the HRTs were changed to 12, 8 and 6 hours respectively. The treatment duration at each temperatures from 5 to 25 °C of different HRTs is presented in Table 3-4.

The reactors were started at 25 °C with a COD concentration of 1000 mg/L and HRT of 24 hour. During this stage the reactors were operated about 65 days until reactors reach stable conditions. The reactors HRT were then changed to 24, 16 and 12 hours HRT for

Table 3-4. Duration of Experimental Studies

Experiment	Date	Reactors (HRT, h)	Number of Days
<u>Startup, 25 °C</u>			
1000 mg/L COD	May 5, 1994-July 10, 1994	1(24), 2 (24), 3 (24)	65
1000 mg/L COD	July 10, 1994-Aug. 15, 1994	1(24), 2(16), 3(12)	35
1000 mg/L COD	Aug. 15, 1994-Sept. 7, 1994	1(12), 2(8), 3 (6)	22
600 mg/L COD	Sept. 7, 1994-Oct. 15, 1994	1(24), 2(16), 3(12)	38
600 mg/L COD	Oct. 15, 1994-Nov. 8, 1994	1(12), 2(8), 3(6)	23
<u>20 °C</u>			
600 mg/L COD	Nov.8, 1994-Jan.. 12, 1995	1(24), 2(16), 3(12)	64
600 mg/L COD	Jan. 12, 1995-Feb.2, 1995	1(12), 2(8), 3(6)	21
<u>17.5 °C</u>			
600 mg/L COD	Feb. 2, 1995-March 15, 1995	1(24), 2(16), 3(12)	43
600 mg/L COD	March 15, 1995-April 5, 1995	1(12), 2(8), 3(6)	20
<u>15 °C</u>			
600 mg/L COD	April 15, 1995-July 5, 1995	1(24), 2(16), 3(12)	90
600 mg/L COD	July 5, 1995-Aug. 3, 1995	1(12), 2(8), 3(6)	28
<u>12.5 °C</u>			
600 mg/L COD	Aug. 3, 1995-Oct. 10, 1995	1(24), 2(16), 3(12)	67
600 mg/L COD	Oct. 10, 1995-Nov. 5, 1995	1(12), 2(8), 3(6)	25
<u>10 °C</u>			
600 mg/L COD	Nov. 5, 1995-Dec. 15 1995	1(24), 2(16), 3(12)	40
600 mg/L COD	Dec. 15, 1995-Jan. 12, 1996	1(12), 2(8), 3(6)	27
<u>7.5 °C</u>			
600 mg/L COD	Jan. 12-Feb.19 1996	1(24), 2(16), 3 (12)	38
600 mg/L COD	Feb.19-March 12, 1996	1(12), 2(8), 3 (6)	22
<u>5 °C</u>			
600 mg/L COD	March 12-April28	1(24), 2(16), 3(12)	47
600 mg/L COD	April28-May26	1(12), 2(8), 3 (6)	28

reactor 1, reactor 2 and reactor 3 respectively and operated 35 days at the same temperatures. After obtaining the equilibrium data (as defined by consistent gas production and quality, COD removal efficiency, and VFA concentrations), the HRTs were changed subsequently to the lower level of 12, 8 and 6 hours, and operated 22 days.

After obtaining equilibrium conditions, the substrate COD concentration was changed from 1000 mg/L to 600 mg/L, and kept constant throughout the experiment. The operating temperature was first lowered from 25 to 20 °C, and after then lowered from 20 to 5 °C in steps of 2.5 °C. Subsequent temperature changes were accomplished by lowering the temperature by 0.5 °C every two days. The gradual lowering of the temperature was used to ensure that granules would not be washed out of the system while the microorganisms were acclimating to the lower temperature. Each bioreactor was operated at two HRTs at each temperature. First, each of the three reactor were operated at the desired temperature with desired HRTs of 24, 16 and 12 hours. After collecting steady-state data at that temperature, the HRTs were changed to their respective lower value. The HRTs were changed slowly, at least in more than three times of HRT so that excessive biomass washout would not occur. Between each subsequent data point, the reactors were operated for a considerable time at the same HRT and temperature to ensure that the data reflects true steady-state conditions.

With the dropping of temperature and lowering the HRTs, the performance of the reactors dropped slowly. During the temperature change from 17.5 °C to 15 °C, the reactors took longer time (90 days) to come in stable operation, which might be due to a change from mesophilic to psychrophilic conditions.

The pumps used for feeding and decanting were constant-speed peristaltic pumps (previously described). Therefore, in order to achieve the desired feeding and decanting volumes in different temperatures, the feed, decant, settle, and react times of the ASBR were changed accordingly. The volume of substrate fed and decanted during each 4 hour sequence of operation, along with other operational variables are shown in Table 3-5.

For the operation of 12. and 8 hour HRT, the decanted and feeding time were changed accordingly, which reduced the react time 3.42 and 3.3 hours for reactors one and two. But for the operation of 6 hour HRT of reactor three, the cycle time was reduced from 4 hour to 2 hour to restrict the biomass washout and unstable conditions.

Table 3-5. ASBR sequencing characteristics used

	Reactors (HRT, hrs)		
	1(24)	2(16)	3(12)
Number of sequences per day	6	6	6
Length of sequence, hours	4	4	4
Volume of feed per sequence, liters	1	1.5	2
Volume of feed per day, liters	6	9	12
Volume decanted per sequence, liters	1	1.5	2
Volume decanted per day, liters	6	9	12
Length of feeding time, min	4.6	8.1	9.8
Length of settling time ^a , min	7	7	7
Length of decanting time	4.8	7.2	10.4
Length of react time, hours	3.7	3.63	3.55

^aSettle time is changed a little to accommodating temperature effects

F. ASBR Mixing

Mixing of the ASBR was conducted on an intermittent basis throughout the study. Just after the feeding phase of the cycle, the gas recirculation mixers were turned on to achieve intimate mixing between the substrate and the biomass. The mixtures were turned on for 30 seconds every 30 minutes during the react phase to release any entrapped gas bubble

within the ASBR and to provide adequate contact between the substrate and the biomass. Mixing was not performed during the settling phase, 7 minutes before the start of decant phase. There was no mixing during the decant phase. The mixing intensity was maintained variable depending on the loading rate and biomass level so that excessive mixing would not break the granules, but adequate contact between the biomass and substrate occurred.

G. Laboratory Analyses

To determine the performance of the reactors, several parameters were frequently analyzed. The decision of whether to change the temperature, the COD loading rate and the HRT was based on these operational parameters. The type of data and the frequency of data collection are shown in Table 3-6. The data were collected when the ASBR system had reached quasi-steady state conditions. Quasi-steady state, or equilibrium condition is defined here by consistent gas production rate and composition, consistent COD removal efficiency, and VFA concentrations.

1. pH (Hydrogen Ion Concentration)

One of the most important operating parameters of an anaerobic system is pH. Although acidogens and acetogens are able to survive over a wide range of pH, methanogens can generally grow only within a pH range of approximately 6.5 to 7.8, optimally 6.8 to 7.2. It was, therefore, necessary to monitor the pH daily for any significant deviation from near-neutral pH conditions. Changes in the pH towards acidic conditions, indicates a potential imbalance in the reactors. A drop in pH would indicate an accumulation of volatile acids and inhibition of methanogenic bacteria.

Samples of the effluent from the ASBRs were collected in beakers. pH measurement were made as soon as possible after the samples were withdrawn from the reactor with little or no agitation to minimize the loss of dissolved carbon dioxide. The pH was measured using a Cole-Parmer model 05669-20 pH meter (Cole-Parmer Company, Chicago, IL) which was calibrated according to the standard method (1995).

Table 3-6. Observed Parameters

Parameter	Method	Frequency
1. pH	pH meter	Daily
2. Total Alkalinity	Standard	Once a week
3. Volatile solids	Standard	Once a week
4. Chemical Oxygen Demand (COD)	Standard	Once a week, 3 times per data point
5. Biochemical Oxygen Demand (BOD ₅)	Standard	Once per data point
6. Solids SS (MLSS, TSS) VS (MLVSS, TSVS)	Standard	Once a week
7. Gas Analysis		
Production	Read gas meter	Daily
Composition	GC Analysis	Once a week
8. Sulfate and Sulfide ^a	Standard	Once per data point
9. Granulation		
Size ^b	AIA analysis	Once per data point
Composition ^c	SEM, TEM	Once per data point
10. Activity Tests ^d	Acetate /Propionate Substrate	Variable

^a Tests performed in ISU Analytical Laboratory Services

^b Tests performed in ISU civil Engineering Materials Laboratory

^c Tests performed in ISU Bessey Microscopic Facility

^d Tests performed on selected granules

2. Alkalinity

The alkalinity of a water or wastewater is its quantitative capacity to neutralize strong acid to a designated pH. Alkalinity in the anaerobic systems is an important parameter in that volatile acids produced by acidogenic and acetogenic bacteria tend to lower reactor pH. Sufficient alkalinity is necessary to maintain the pH of the system at or near 7.

In many wastewaters, alkalinity is a function of the carbonate, bicarbonate, and hydrogen oxide content. In waters containing significant phosphates, silicates, and borates, the total alkalinity is also a function of these species. In a typical anaerobic system, the hydroxide and carbonate concentrations are negligible as compared to the bicarbonate concentration. Therefore, total alkalinity is essentially equal to bicarbonate alkalinity (plus borate, phosphate, and silicate alkalinity, if applicable).

The total alkalinity of the ASBRs effluents was measured using the procedure outlined in standard method [1995]. During each test, 25 ml effluent samples were used. The samples were titrated using 0.1N sulfuric acid to an end point pH of 4.5. The total alkalinity was calculated using the equation:

$$Alk = (A)(N)(50,000) / V \quad 3-1)$$

where.

Alk	= total alkalinity, mg/L as CaCO ₃
A	= volume of standard acid used, ml
N	= normality of standard acid, eq/L
V	= volume of effluent sample titrated, mL
50.000	= equivalent weight of CaCO ₃ , mg/eq

The accuracy of the method used for determining alkalinity is said to depend on sample characteristics. However, deviation of the results from actual value is said to range from ± 1 mg/L to ± 9 mg/L [Standard Method, 1995].

3. Volatile Fatty Acids

Volatile fatty acids (VFA) are classified as water-soluble fatty acids (up to six carbon atoms) that can be distilled at atmospheric pressure. They are removed from aqueous solution by distillation despite their high boiling points due to their high vapor tensions.

The VFA concentration of an anaerobic wastewater is a measure of the balance that exists among the acidogenic, acetogenic, and the methanogenic bacterial populations. VFAs arise from the oxidation of sugars, alcohols, lipids, and long- chain fatty acids by the acidogenic and acetogenic bacteria. Under proper anaerobic digestion, the acetogenic bacteria convert the VFA to acetic acid, and the methanogenic convert acetic acid to methane and carbon dioxide. As such, high VFA concentrations signal an upset system in which the methanogenic bacteria are not keeping pace with the acidogenic and acetogenic bacteria.

The VFA test was conducted according to the standard method #504C [Standard Method, 1995]. Effluents from the reactors were relatively low in solids, therefore, centrifugation was not required. One -hundred mL of the effluent sample was placed in a 500 mL distillation flask, together with 100 mL of distilled water, 5 mL of concentrated H_2SO_4 , and several glass beads. The samples were then distilled at a rate of approximately 5 mL/minute, and 150 mL of the distillate were collected. The distillate was titrated to the phenolphthalein endpoint with 0.1 N NaOH. The VFA concentration was determined assuming that 100% of volatile acids were collected, using the following equation:

$$VFA = (A) * (N) * (60,000) / V \quad 3-2)$$

where,

VFA = conc. of volatile acids, mg/L as acetate

A = volume of NaOH used, mL

N = normality of NaOH solution, eq/L

V = volume of effluent, 100 mL

60,000 = equivalent weight of acetic acid, mg/eq

4. Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) of a wastewater is defined as the amount of a strong oxidizing agent required to chemically oxidize the organic matter present in a given sample, expressed as potassium dichromate. The test was performed using the closed Reflex Titrimetric Method #508 [Standard Method, 1995].

The influent and effluent from the ASBRs were tested for their respective COD values, from which the COD removal percentages could be calculated by the following equation. Additionally, both soluble and total COD in the effluent were determined. Soluble COD is defined here as the COD passing a 0.45 μ M filter. Both soluble and total COD removal percentages, however, were based on the total influent COD concentration.

$$COD\ Removal, \% = (COD_i - COD_e) \times 100 / COD_i \quad 3-3)$$

where, COD_i = influent COD concentration, mg/L

COD_e = effluent COD concentration, mg/L

The difference between soluble COD and total COD removal percentages is generally a measure of the COD of the solid in the effluent.

The COD test was conducted as follows. Effluent and influent samples were collected from the ASBRs. The samples were mixed well on a magnetic stir plate, and the appropriate volume of each sample was withdrawn and placed into separate 100 mL volumetric flasks. These flasks were filled to the 100 mL mark with deionized and distilled water. Approximately 30 mL of the diluted effluents samples were then vacuum filtered through 0.45 μ M glass fiber filters (Fisherbrand G4, Fisher Scientific, Pittsburgh, PA). The filtrate was used in determination of the soluble COD concentration of the effluent. Five mL of each sample was then withdrawn and placed in a 30 mL screw-top test tube. Three mL of 0.1 M $K_2Cr_2O_7$ were then dispensed into the test tubes, followed by 7 mL of concentrated sulfuric acid. Table 23 lists the chemical composition of the various reagents used in the COD test. The test tubes were then capped with phenolic caps (corning, Inc., Corning, NY) and placed

in a 150 °C oven for two hours. At a minimum, all samples were tested in duplicate.

Additionally, four 5 mL samples consisting of distilled and deionized water were also tested in the manner previously outlined. Two of these were placed in the oven with the other samples, called blanks, and two were set aside and not placed in the oven, called standards.

After heating in the oven, the COD samples were removed and allowed to cool to room temperature. After cooling, two drops of a ferroin indicator (Table 3-7) were put into each sample. The samples were then titrated with 0.1 N ferrous ammonium sulfate (FAS) to the ferroin endpoint. The standard were used to determine the precise normality of the FAS solution. The blanks provided a measure of the COD of the dilution water. The COD of a given sample was then calculated as shown in Equation:

$$COD = (A - B) * (N) * (DF) * (8000) / V \quad 3-4)$$

where, COD = chemical oxygen demand of the sample, mg/L

A = volume FAS used for blank, mL

B = volume FAS used for sample, mg/L

V = volume of sample, 5 mL

N = normality of FAS solution, eq/L

DF = dilution factor of the sample

8000 = equivalent weight of oxygen, mg/eq

Since the maximum COD measured by this method is 450 mg/L for 5 mL samples, the samples normally required dilution so that diluted sample had a COD less than 450 mg/L. "DF" in the above equation is the diluted sample volume divided by the undiluted sample volume. For example, if 20 mL sample was diluted to 100 mL mark of the volumetric flask, the dilution factor is 100 divided by 20 i.e., 5. The normality of the FAS solution (N) is calculated using that standards. The dichromate solution is 0.1 normal. The FAS solution is

also made to 0.1 N, but this solution photo-degrades over time so that the active normality of the FAS solution is somewhat less than 0.1, as given in equation:

$$N = (3.0 * 0.1) / D \quad 3-5)$$

where, N = actual normality of FAS, eq/L (0.1)

D = volume of FAS used for standards, mL(>3.0)

3.0 = theoretical volume of FAS required for standards if FAS was exactly 0.1N, mL

0.1 = theoretical normality of FAS solution, eq/L

Table 3-7. Reagents used in the COD test

Reagent Solution	Component Name	Concentration
COD Acid	H ₂ SO ₄ (Concentrated)	36.0 N
	Ag ₂ SO ₄	10.0 g/L
Oxidizing Solution	K ₂ Cr ₂ O ₇	4.913 g/L
	HgSO ₄ (conc.)	33.33 g/L
	H ₂ SO ₄	167.0 mL/L
	H ₂ O	833.0 mL/L
Ferrous Ammonium Sulfate	Fe(NH ₄) ₂ .6H ₂ O	39.2 g/L
	H ₂ SO ₄ (conc.)	20.0 mL/L
	H ₂ O	980.0 mL/L
Ferroin indicator	1.10-phenanthroline	14.85 g/L
	FeSO ₄ .7H ₂ O	6.95 g/L
	H ₂ O	1.0 L/L

5. Solids (Effluent Suspended Solids)

The effluent samples taken for COD and VFA determination were also analyzed for total suspended solids (TSS) and volatile suspended solids (VSS) using a procedure similar to 208 D and E of Standard Methods [1995]. Since the ASBR system has internal clarification, the level of TSS is a measure of how well the solids in the ASBR are settling.

A day before the test, the required number of filter papers (Fisherbrand G6, Fisher Scientific, Pittsburgh, PA) were folded in fourths and placed in aluminum weighing dishes. These were ignited at 550 for 20 minutes and then cooled to room temperature in a desiccator for not less than 2 hours. The filters and dishes were then weighed to the nearest 0.1 mg on a Mettler model AM 50 digital scale (Mettler Instrumentation Corporation, Hightstown, NJ) and returned to the desiccator until needed. Just before the solids tests was conducted, the filter paper and dishes were removed from the desiccator. The filter apparatus, consisting of vacuum pump, a 500 mL suction flask, a 4 in porcelain Buchner funnel, and the necessary connecting hoses, was set up. The filter paper was removed from the aluminum weighing dish and placed in the Buchner funnel.

The samples to be tested were placed on a magnetic mixer and a specific volume was withdrawn from the sample with an appropriate pipette. The volume of the sample was dependent on the approximate solids content of the sample, but was between 10 and 25 mL. The withdrawn (and a duplicate) sample was pipetted through the filter, and were then vacuum-filtered until no free water was left on the filter paper. The filter paper was returned to the weighing dish and these were then transferred to an oven and dried at 103 °C for not less than one hour. After drying, the samples were placed in a desiccator until cooled to room temperature and then weighed. The effluent TSS concentration and VSS concentration were calculated using the formulas:

$$\text{Effluent TSS} = (B - A) / V * 10^6 \quad 3-6)$$

$$\text{Effluent VSS} = (B - C) / V * 10^6 \quad 3-7)$$

where, TSS = total suspended solids concentration, mg/L

VSS = volatile suspended solids concentration, mg/L

A = initial weight of dish and filter(before filtering), grams

B = initial weight of dish and filter (after drying), grams

C = weight of dish and filter (after ignition), grams

V = volume of sample, mL

10^6 = conversion from g/mL to mg/L

6. Solids (Mixed liquor Suspended Solids)

The mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) are a measure of the suspended solids in an operating ASBR. The MLSS is the concentration of all solids with an effective diameter greater than $0.45 \mu\text{M}$, whereas the MLVSS measures only the volatile fraction greater than $0.45 \mu\text{M}$. The MLVSS is a better measure of viable biomass, although neither MLSS nor MLVSS is a true measure of the amount of active microbes present. MLVSS is typically used to approximate the organic solids present in a reactor. The difference between volatile and organic matter is generally due to the volatilization of some inorganic compounds, most notably inorganic carbonates. That is, some inorganic carbonates are volatilized in the test and do not show up as fixed solids but rather as volatile solids. Therefore, equating volatile residue with organic residue would be in error. Despite the difference in the two parameters, volatile solids were used to approximate the viable fraction because of the relative ease of the MLVSS test as compared to the total organic carbon test.

The MLSS and MLVSS tests were conducted as TSS and VSS suspended solids tests, but mixed liquor was used instead of effluents. The mixed liquor was taken from 3 ports

during mixing for representable sample. The same equations 3-6 and 3-7 were used to determine the MLSS and MLVSS.

7. Biogas Production and Composition

During methanogenesis, stabilization of the waste occurs due to the liberation of methane. Little reduction in COD of the materials occur in the hydrolysis and acedogenic stages, since the degradation products in a given waste are not liberated from the system. Therefore, measurement of methane production is a useful tool for determining the degree of COD stabilization of waste.

The COD-equivalent of the methane produced from anaerobic degradation of an organic substrate can be calculated by the following equation.



The COD-equivalent of methane is, therefore, 2 moles of COD (oxygen) per mole of methane produced. Equivalently, the COD-equivalent is 0.35 L CH₄/g COD removed, or 5.61 ft³ CH₄/lb COD removed (all values are in standard temperature (0 °C) and pressure (1 atm.)). Analysis of the composition of biogas produced from a reactor can then be used to determine the volume of methane produced, which enables a calculation of COD stabilized in the reactor. Analysis of the composition of biogas produced from a reactor and sulfate reduction can then be used to determine COD stabilization or vice versa.

The daily biogas was measured with tip meter. The meter reading was taken daily at ambient pressure and temperature. The daily biogas volume were then expressed as standard biogas production at 1 atm. and 0 °C.

The biogas was analyzed using a gas chromatograph (GC) equipped with a thermal conductivity detector. The operating condition and parameter for the GC analyses are shown in Table 24. The gas samples were obtained from the gas sampling ports located in the gas line of the reactor system, using a 1 mL syringe (Hamilton Company, Reno, NV) equipped

with 2 in-long Metal Hub Needles (Alltech Associates, Inc., Deerfield, IL). The syringe was purged with biogas three times for collecting the sample. The volume of the sample 0.9 mL. More than 0.9 mL samples were drawn initially, but prior to insertion into GC, some of the samples were purged out such that only 0.9 ml sample was fed into GC. The purging was done to prevent any accumulated air on the syringe from being injected into the GC.

The data from the gas analyses were collected and analyzed using the Baseline 810 Chromatography Workstation software package (Waters Dynamic Solutions, Division of Millipore, Ventura, CA). The output from the analyses listed the percentages of methane, carbon dioxide, and nitrogen in the biogas. The nitrogen percentage in the biogas was actually a measure of the amount of air in the system. Theoretically, there should be no air in the system, since oxygen is toxic to anaerobic organisms. However, a small amount of air will enter the reactor due to the solubility of these gases in the feed substrate. In a well-operated system, oxygen entering the reactor through the influent will rapidly be scavenged by facultative organisms and other oxygen-demanding substances. Therefore, the amount of nitrogen in the biogas was reflective of the air-tightness of the system.

8. Automated Image Analysis

Automated Image Analysis (AIA), coupled to an Olympus BH-2S upright, transmitted light microscope, was used to monitor the changes in biomass morphology and size in the ASBRs. The particle image to be analyzed by AIA is obtained through a TV/video camera mounted on a microscope and input to the system as a digitized image. The digitized image can be mathematically manipulated to enhance the desired feature appearance by using the system editorial functions such as small feature enhancement, erosion, dilation, and so on. Once the particle image is extracted from the surrounding background, it can be sent to the system computation unit for the final report. The report includes the particle counts, cover area density, particle orientation (degrees), particle area distribution plot, particle size distribution plots, and so on.

Table 3-8. Gas chromatograph operating conditions and parameters.

Parameter	Specification
Model	Glow-Mac 69-350
Column	6 ft x 3 mm ID stainless steel
Packing	porpak Q, 80/100 mesh
Temperature	Ambient
Carrier Gas	Helium
Flow Rate	60 mL/min
Pressure	60 psig
Detector	Thermal conductivity
Temperature	150 °C
Bridge current	150 mA
Sensitivity	10 mA
Injection Point temperature	100 °C
Outlet temperature	70 °C
Sample size	0.9 ml
Standard gas	
methane, %	70
carbon dioxide, %	25
nitrogen, %	5

Image processing used in this study consisted of five steps: magnification calibration, setting gray levels for analysis, image acquisition, editing displayed image, and frame analysis. The first step of image processing is to calibrate the magnification of the optical system (microscope-AIA combination) using a 0.5 mm ruler and record the magnification into the computation program. The optical system can provide magnification ranging from 10 to 60x. A pertinent magnification of the optimal system can be selected by the operator

depending on the particle size of the sample. Generally, for particle sizes ranging from 0.1 to 3 mm, a magnification range of 10 to 20x is adequate.

The second step is setting gray levels for analysis. The AIA identifies and measures the objects based on the contrast between the object and the background, which is represented by the level of gray in the field of view. There are 256 levels of gray, from black at level zero, to white at level 256, to be used according to the characteristics of particle. This option allows the operator to set upper and lower thresholds for gray level. When a particle image is threshold properly, the AIA can correctly identify, count, and size the particle. In the study of anaerobic particles, the gray levels are easy to set because the particle is quite dark and distinct compared to the surrounding background. The set level for anaerobic sludge used in this study was low level 0 and high level 50. After setting the threshold gray level, the image is acquired from the video camera. The AIA directs the video signal to the monitor. Adjustment of the light contrast and focus are needed in this stage in order to gain the most clear image. The image then can be frozen for processing.

For editing the image, first the small feature enhancement is processed. This operation amplifies the gray level at locations of rapid change, where the images of small features are detected and presented on the screen. Next, erode and dilation options are sequentially performed to lead a more accurate feature count during analysis. The processed image frame is finally sent to AIA computation unit for analysis, and then the results are stored to disk for printing. The output from the software included histogram of the size distributions and total mass distribution, as well as average characteristics of the entire sample.

A sample cell of plexiglas and glass was made for holding the sludge sample under the microscope. Approximately 25 particles suspended in water were placed in specially prepared slides. The slides consisted of an open well 25 mm in diameter and 2 mm deep. Distilled water was used to fill the well to avoid stray reflections from a meniscus either above or below the top of the well to level to avoid stray reflections from a meniscus either

above or below the top of the well. Images were digitized at 640 x 480 pixels and stored in TIFF format for later analysis. Six frames were collected per sample for a total, of around 150 particles per sample.

During analysis, some manual editing of the binary image was necessary to separate contacting particles. The area and perimeter were measured for each particle. A variety of measurement were then calculated from these basic measurements including area-weighted diameter (the diameter of a particle with the same area). Size distributions were calculated showing particle count, area, and volume fractions as a function of size using both logarithmic and linear scales. Mean diameter was calculated on a number of bases: ordinary mean diameter, area-weighted diameter (i.e., $\sum d^3 / \sum d^2$), and geometric mean diameter as

$$\sum (d_1 \times d_2 \times d_3 \dots d_n)^{1/n}$$

9. Scanning and Transmission Electron Microscopy

Scanning and Transmission Electron microscopy (SEM and TEM, respectively) analyses were performed on the granules of ASBR to observe the morphology and structure of granules. Direct observation of the bacterial consortium within each granule allowed a qualitative estimation of similarities and differences among the granules under various conditions. In other words, if one experiment yielded a granule mainly consisting of cocci, while the granules from other experiment were composed of mainly rods, one plausible explanation for this is that the experimental enhancement method utilized in those experiments physically selected for those type of bacteria.

It was also desired to observe the homogeneous or heterogeneous distribution of the bacterial consortium of the granules. That is, the use of electron microscopy could elucidate whether the granule consisted of layers, with each layer consisting of different bacterial group, or if the granules were homogenous throughout, with all groups of bacteria present in each part of the granule.

The SEM and TEM analyses were conducted at the Bassey Microscopy Facility, located at Iowa State University. Sample from each temperature and Loading rate were taken to this facility, and all preparatory work and analyses were performed by the Bassey staff.

The procedure for processing sample comprised of several steps. First, the bacteria were fixed in 4% glutaraldehyde, 0.05M cacodylate buffer were added for maintaining pH 7.2, and stored overnight at 4 °C. The next day, the samples were washed in the same buffer three times for 10 minutes. The sample then fixed in 1% Osmium Tetroxide (OsO_4), using same buffer for 1 hr at temperature 4 °C. The sample washed three times with the same buffer again for 10 minutes. The samples were dehydrated in an ethanol series to 100% (50%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 100%), and then infiltrated to 100% propylene oxide as an intermediate fluid from 100% ethanol.

For the SEM, critical point dried from 100% ethanol in CO_2 , mounted on brass discs and sputter coated in a polaron E5100 with platinum /palladium target (60:40) and observed with JEOL JSM-35 SEM at 20 kV. But for the TEM, the infiltrated specimens were embedded in epoxy resin and cured for 24 hours in 60 °C oven. The specimen were then cut with a diamond knife on a Reichert Ultracut E ultramicrotome and placed on copper grids for post-staining with uranyl acetate and lead citrate. The specimen photographed on a JEOL 1200 EX scanning transmission electron microscope.

10. Elemental Analysis of Granules

Several reports in the literature have cited significant amounts of specific inorganic compounds or elements in granules, which effect the structure of granules. The granules size, composition and structure have been evaluated to determine the change of temperature and loading rate.

The elemental and chemical analyses of the granules was conducted at the Materials Testing Laboratories of the Civil and Construction Engineering Department by X-ray analysis.

11. X-ray Analysis

One slide per sample allowed to air-dry following collection of the image for image analysis in order to perform energy-dispersive X-ray analyses. Samples were examined in a Hitachi model 2460N low-vacuum scanning electron microscope equipped with a link Isis x-ray analysis system with a Ge light-element X-ray detector. An accelerating voltage of 20 KV was used at a beam current of about 0.5 nA. A residual atmosphere of 40 Pa of helium was used to permit examination of the uncoated particles.

Spectra were collected for four typical particles per sample using a raster scan at a magnification of 200-400 times to cover much of the surface of the globule without exciting the substrate. Spectra were collected for up to 150 seconds per particle at a count rate of about 1500 particles per second. They show the sensitivity of the system to light elements and the difference in composition that was encountered between samples.

Estimates of concentration were made using the standardless x-ray analysis package provided with the link analyzer. An aluminum sample was used as an x-ray intensity reference to calibrate the spectra for the use of the stored element profiles. Concentrations were calculated for O, Na, Mg, Al, Si, P, S, Cl, K, Ca, Fe, Co, Ni, and Zn. Since the analyses were performed without standards, there is a large uncertainty in the results ($\pm 25\%$). However, since all samples were analyzed under similar conditions, the trend in concentrations between samples should be valid. Small amounts (e.g., 0.05%) of the metallic elements were definitely determined to be present in some samples while not in others. The detection level for most elements was on the order of 0.02%.

CHAPTER 4**ASBR TREATMENT OF DILUTE WASTEWATER AT PSYCHROPHILIC TEMPERATURES**

A paper presented in the 69th WEFTEC conference

Gouranga C. Banik, Timothy G. Ellis, Richard R. Dague

ABSTRACT

Anaerobic treatment of dilute wastewater was studied using three, laboratory-scale anaerobic sequencing batch reactors (ASBRs), each with an active volume of six (6) liters. The reactors were fed a synthetic substrate made from non-fat dry milk supplemented with nutrients and trace metals. The COD and BOD₅ of the feed was 600 mg/L and 285 mg/L, respectively. Steady-state performance data were collected at reactor temperatures of 25, 20, 17.5, 15, 12.5, 10, 7.5 and 5 °C over a period of two years. Hydraulic retention times (HRTs) were maintained at 24, 16, 12, 8 and 6 hours. Steady- state removal efficiencies were evaluated for the various conditions.

Results showed that the ASBR process was capable of achieving in excess of 90% soluble COD and BOD₅ removal at temperatures of 25 °C and 20 °C at all HRTs. At the low temperature of 5 °C and the six hour HRT, soluble COD and BOD₅ removals were 62% and 75%, respectively. At the intermediate temperatures from 20 °C down to 5 °C and HRTs between 24 and 6 hours, removal of soluble organics ranged between 62 and 90 % for COD and 75 and 90 % for BOD₅. In all cases, solid retention times (SRTs) were high enough to maintain good performance. It is concluded that the ASBR has unique characteristics that enable efficient removal of organics when treating dilute wastewaters at low temperatures.

KEY WORDS

Anaerobic treatment, ASBR, dilute wastewater, low temperature, granular biomass

INTRODUCTION

Several high-rate anaerobic processes have been developed since the early 1950s. These include the anaerobic contact process, the anaerobic biofilter, the upflow anaerobic sludge blanket (UASB), the fluidized-bed, and, most recently, the anaerobic sequencing batch reactor (ASBR). Common experiences of the past indicate that anaerobic processes are not capable of achieving efficient organic removals when treating wastewaters at temperatures below the mesophilic range (25-40 °C) (Lin et al. 1987; Sutter et al. 1985; Viraraghavan et al. 1991).

The performance of all biological treatment processes is affected by temperature. Lower temperatures result in lower rates of substrate removal and biomass growth. Endogenous decay rates are lower at reduced temperatures, which offers the possibility of increasing the biomass concentration in the reactor, offsetting the reduced specific substrate removal rates (Dague et al. 1970).

Modern "high rate" anaerobic reactors are typically applied to the treatment of high-strength wastewaters (COD > 1000 mg/L) at temperatures in the mesophilic range. To date, practically all full-scale applications of anaerobic treatment are restricted to wastewaters with a temperature exceeding 18 °C. Many wastewaters have temperatures lower than the mesophilic range (<25 °C) and normally require heating for efficient treatment (Maaskant et al. 1983).

Kennedy and van den Berg (1982) evaluated the effects of temperature (10-35 °C) and overloading on the performance of anaerobic fixed film reactors digesting bean blanching wastes. They found that for lowering temperature from 35 to 10 °C, loading rate would be reduced by 75% for similar performance. Sharp decrease of the rate of anaerobic treatment performance was observed when manure was digested at a temperature below 20 °C (Parshina et al. 1994). The anaerobic filter at 20 °C achieved higher levels of performance in comparison to 10 and 5 °C, treating septic tank effluent (Viraraghavan et al. 1991). They

found that the reactors operating at 5 °C was the most affected by HRT changes. In contrast, the expanded granular sludge blanket (EGSB) performed well in treating VFAs at a temperature range of 10-12 °C (Rebac et al. 1995). Safley and Westerman (1990) suggested that reasonable methane yields are possible for anaerobic digestion at low temperatures if digesters loading rates are appropriately reduced.

Lower substrate concentrations in the reactor lead to lower removal rates, in accordance with Monod kinetics (Monod, 1949). Low substrate concentrations combined with low temperatures (psychrophilic) result in a deterioration of process performance, as compared to the case of high substrate concentration and higher temperatures. The ASBR has been under development by Dague and co-workers at Iowa State University for several years (Sung and Dague, 1992, 1995; Wirtz and Dague, 1996). These studies have shown that the ASBR performs well in treating medium and high strength liquid wastes of industrial and agricultural origin in the mesophilic temperature range.

The COD values of many industrial and domestic wastewaters are below 1,000 mg/L and many of these dilute waste streams are also low in temperature (Kato et al. 1994). Successful anaerobic treatment of such wastewaters using this process without the addition of heat would be a significant achievement that could have a major impact in reducing the cost and energy requirements for wastewater treatment.

Early research by Dague et al. (1966) found that a batch fed and decanted anaerobic reactor could achieve high and equal levels of substrate removal at temperatures of either 35 °C or 25 °C. The system was capable of compensating for the decrease of temperature from 35 to 25 °C by increasing the biomass concentration. Temperatures achieved in the present research were much lower (5 °C) than the earlier research. It is believed that the ASBR has unique characteristics that enable achievement of high levels of substrate removal at short hydraulic retention times (HRT) when treating dilute wastes at low temperatures.

The operating principles of the ASBR have been described by Sung and Dague (1995). In operation, the ASBR sequences through four cycles: feed, react, settle and decant. This results in a variable substrate concentration in the reactor, alternating from high concentrations during and immediately after feeding to low substrate concentrations at the end of the react cycle, just before biomass settling and effluent decanting. The high food to microorganism (F/M) ratio just after feeding results in high rates of substrate removal. However, just prior to biomass settling, the F/M ratio is low and gas production is also low, leading to ideal conditions for biomass settling and retention in the reactor. Previous studies have shown that the ASBR selects for granular sludge (Sung and Dague, 1995). This enhances the ability of the reactor to retain biomass, leading to long solids retention times (SRT) and efficient waste treatment.

Preliminary studies by Udemé and Dague (1995) on the ASBR treatment of a synthetic substrate (non-fat dry milk) with COD concentrations ranging from 1000 to 400 mg/L at temperatures ranging from 35 to 15 °C showed promise of efficient treatment.

The purpose of the research reported here was to evaluate the performance of the ASBR in the treatment of a synthetic waste with a COD concentration of 600 mg/L ($BOD_5 = 285$ mg/L) at temperatures ranging from 5 °C to 25 °C (psychrophilic range) at various HRTs of 24, 16, 12, 8 and 6 hrs.

MATERIALS AND METHODS

The details of the materials and methods are explained in Chapter 3 of this dissertation. The properties of substrate (non-fat-dry-milk) and trace minerals are also explained in Chapter 3.

Reactors

Three identical ASBR reactors of 6 L effective volume were used for this study. The reactors were made by using 12 mm thick Plexiglas and are cylindrical in shape. The ASBR experimental setup is shown in Figure 4-1.

Substrate

As in the previous fundamental research on the ASBR at Iowa State, the substrate used in this study is a synthetic waste which consists of non-fat dry milk (NFDM), sodium bicarbonate, and a trace mineral solution. Sodium bicarbonate (NaHCO_3) is used to maintain suitable buffering. Five trace minerals (Fe, Zn, Ni, Co, Mn) are added to the substrate to provide adequate nutrients for the biomass. The properties of substrate (NFDM) and trace minerals were explained by Kaiser et al (1993).

The reactors were fed with the NFDM substrate with a COD concentration of 600 mg/L. The BOD_5 concentration was approximately 285 mg/L. The influent COD

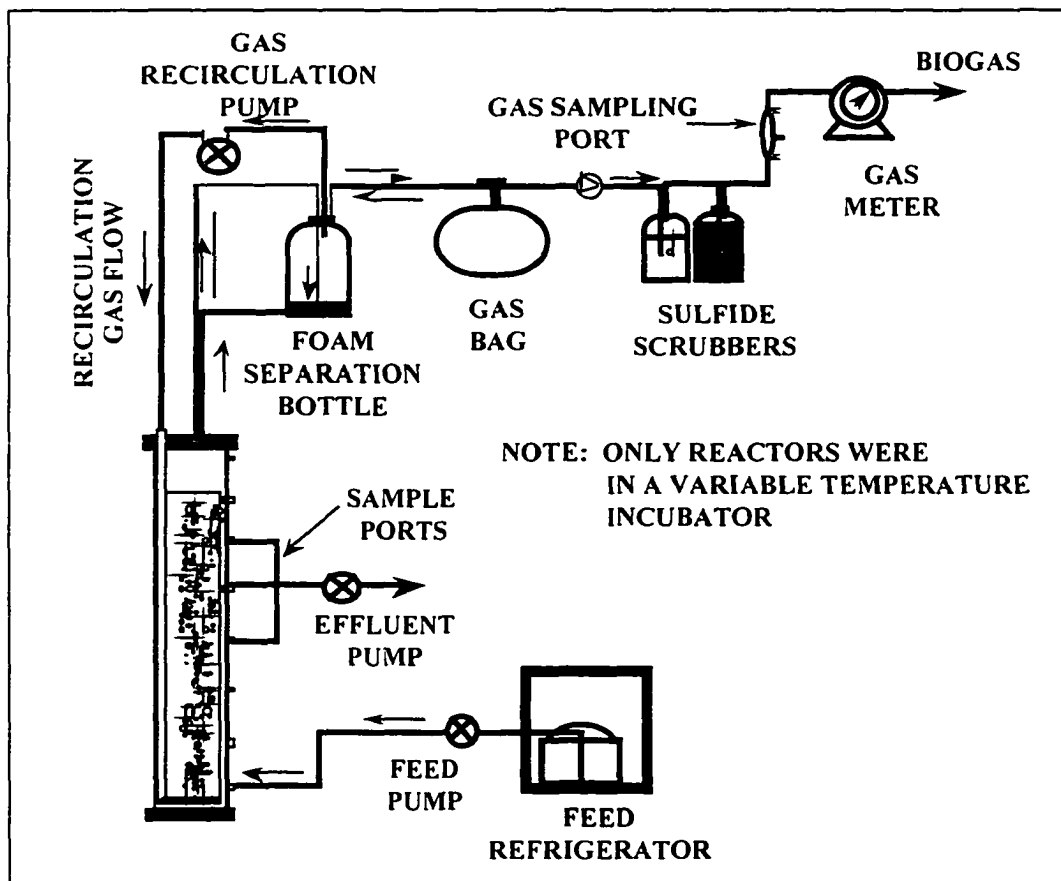


Fig. 4-1 ASBR setup for the dilute wastewater treatment.

concentration was kept constant so that the effect of varying HRT and temperature could be determined independently.

Biomass Seed

All three reactors were inoculated with elutriated mesophilic granular methanogenic sludge obtained from an ongoing ASBR pilot-plant study at the Water Pollution Control Plant at the City of Cedar Rapids, Iowa. Sufficient granular biomass was added at start-up to bring the MLSS to approximately 12 g/L.

Start-up and Operation

Reactor operation started with a substrate COD concentration of 1000 mg/L at 25 °C and at an HRT of 24 hours. Following the achievement of pseudo-steady-state under these conditions, the substrate COD concentration was changed from 1000 mg/L to 600 mg/L. Each reactor was operated at two HRTs at each temperature. The operating temperature was lowered first from 25 °C to 20 °C and then lowered from 20 °C to 5 °C in steps of 2.5 °C. First, the reactors were operated at a specific temperature with HRTs of 24, 16, and 12 hours. After collecting steady-state data at that temperature, HRTs were changed to their subsequent respective lower value. Between each subsequent data point, the reactors were operated for a considerable time at the same HRT and temperature to ensure that the data reflects pseudo-equilibrium conditions. Subsequent temperature changes were accomplished by lowering the temperature by 0.5 °C every two days. This gradual lowering of the temperature was used to ensure that biomass would not be washed out of the system while the microorganisms were acclimating to the lower temperature. The HRTs were also changed slowly to ensure against excessive biomass washout.

Methods

To determine the performance and stability of the reactors, pH and gas measurements were recorded every day. Alkalinity, volatile Fatty acids (VFA), COD, solids and gas

characteristics were determined once per week. In each pseudo-steady-state condition, the parameters were determined three times, and the results were averaged to obtain a data point. Analyses for BOD₅, sulfate, sulfide and automated image analysis (AIA) were also done for each data point. *Standard Methods* were used for all of the conducted tests (1995).

RESULTS AND DISCUSSION

Experiments were conducted for a duration of more than two years at hydraulic retention times (HRT) of 24, 16, 12, 8 and 6 hours. At the feed COD concentration of 600 mg/L, this corresponds to COD loading rates of 0.6, 0.9, 1.2, 1.8, 2.4 g/L/day, respectively. All effluent COD values were compared to the influent total COD (TCOD) of 600 mg/L and influent BOD₅ of 285 mg/L to calculate organic removals.

COD and BOD₅ removals were the primary indicators for evaluating the performance of the reactors at various low temperatures and HRTs. Figure 4-2 shows the variation of COD (total and soluble) and BOD₅ at HRTs of 24, 16, 12, 8, and 6 hours at temperatures of 25, 20, 17.5, 15, 12.5, 10, 7.5, 5 °C. The TCOD, SCOD and BOD₅ removals decreased with temperature reductions. The TCOD, SCOD and BOD₅ removals were in excess of 90% at all HRTs at 25 °C. At 20 °C, COD and BOD₅ removal efficiencies decreased, but remained at or above 90% at all HRTs. The decrease in removal rate was much more pronounced in the temperature range from 12.5 to 5 °C than in the upper range of temperatures.

Figure 4-3 demonstrates the TCOD, SCOD and BOD₅ removals for each temperature and HRT, but the difference here in comparison to Figure 4-2 is that removals in respect of TCOD, SCOD and BOD₅ were compared separately. As illustrated in the Figure 4-3, performances based on TCOD, SCOD and BOD₅ removals decreased with the increase in organic loading. All removals decreased sharply from 12.5 to 5 °C in comparison to 25 to 12.5 °C. The SCOD removals are higher than the TCOD removals at each temperatures and

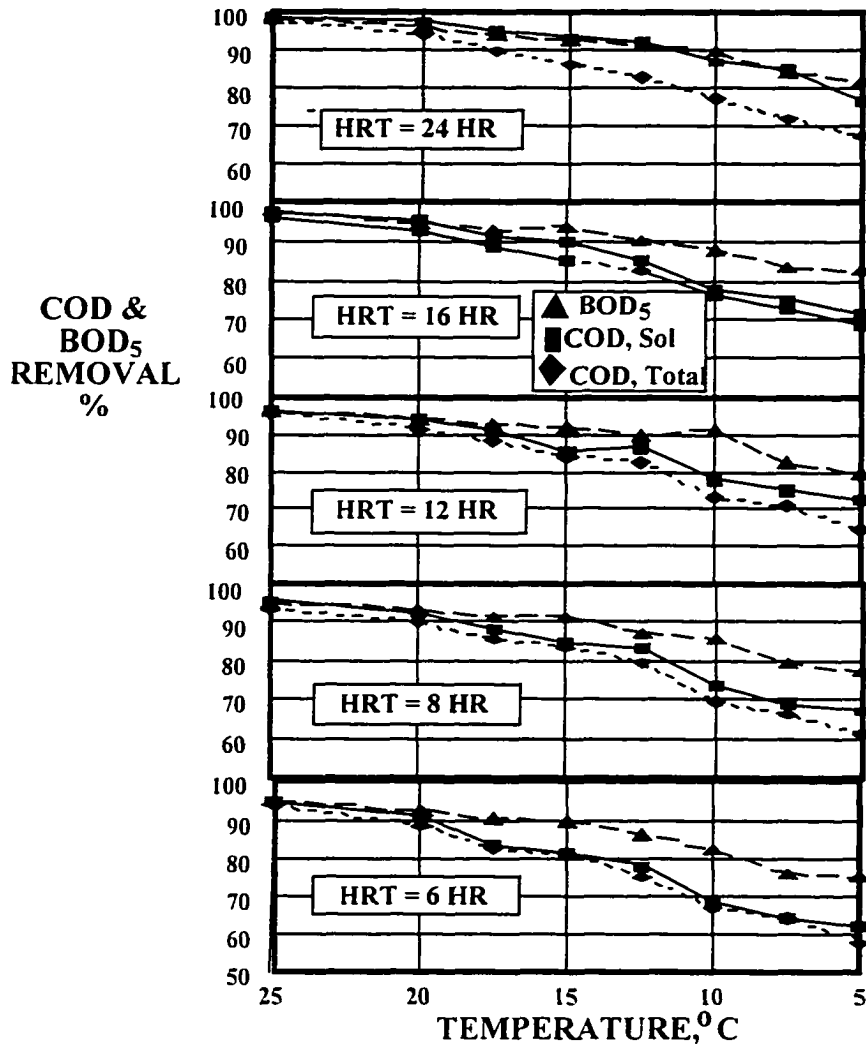


Fig. 4-2. Total COD, soluble COD and BOD₅ removals at various temperatures and HRTs.

HRT as because biomass was not accounted during SCOD measurement . On the other hand, SCOD removals are lower than BOD₅ removals at each temperatures and HRTs.

The possible reason for this is that BOD measures only the biodegradable organics, whereas COD measure the chemically oxidable organics. At 25 °C and 24 hr HRT, all the removals were very close to each other. Performance of the ASBR in relation to TCOD removals was about 58 and 68% for 2.4 and 0.6 g/L/day organic load, respectively at 5 °C.

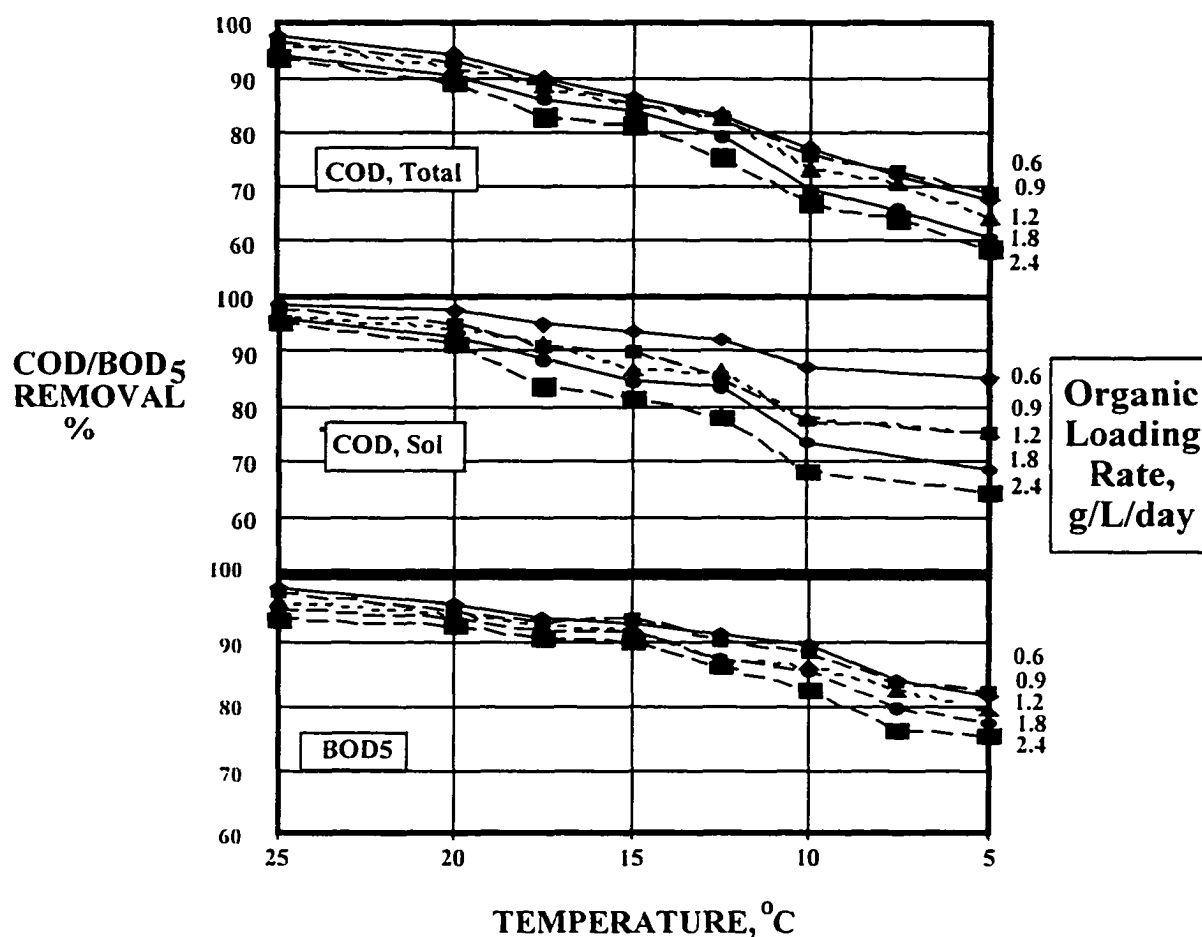


Fig. 4-3. TCOD, SCOD and BOD₅ removals at various temperatures and organic loading rates.

On the other hand, SCOD removals were 62 and 77 percent for the same conditions. But BOD₅ removals were about 75 to 82 percent for the same range of organic load at 5 °C. At a temperature of 15 °C, little below the ambient range of temperature, TCOD and SCOD removals were above 80 percent, but BOD removals was above 90 percent for all loading conditions.

With decreasing HRT, the COD loading rate increased and removal rates decreased. At the relatively higher load (6 hour HRT) the removal rates were much lower than at the 24 hour HRT at all temperatures. The longer HRT allowed greater conversion of the substrate to

biogas at any given temperature. Also, more biomass may be lost at a shorter HRT, resulting in an increased F/M ratio and reduced substrate removals. Although the removal rates were much lower at 5 °C and 6 hours HRT than at the higher temperatures and longer HRT, BOD₅ and SCOD reductions were about 75 and 62 percent, respectively. This clearly demonstrates good performance for the ASBR at this unusually low temperature and short HRT.

Figure 4-4 shows the variation in MLSS and MLVSS with temperature at all HRTs. The MLSS and MLVSS were about 10 g/L and 9 g/L, respectively for all three reactors at 25 °C with a COD concentration of 600 mg/L. At all HRTs, MLSS and MLVSS levels decreased as the temperature declined. The MLSS and MLVSS were reduced more pronounced when temperature was reduced from 25 to 20 °C. This may be due to the lack of acclimation. Below 20 °C, MLVSS were remained fairly constant at all temperatures and HRTs, except 24 hr HRT. The possible reason for the low MLVSS at a 24 hr HRT, was due to the lower synthesis for lower organic loading rate, which did not balance the biomass loss with the effluent. But at the lower HRTs, the decrease in solids levels with declining temperature was less pronounced. This is due to higher biomass synthesis and growth for the higher organic loading at lower HRT. However, at the short HRT there is a greater opportunity for biomass loss in the effluent, since greater volume of liquid is decanted each cycle. These two factors should tend to offset each other. The key is to ensure that the quantity of biomass synthesis and retention in the reactor is greater than the loss of biomass in the effluent.

The variation in effluent volatile suspended solids (EffVSS) and solids retention time (SRT) is illustrated in Figure 4-5 and Figure 4-6 respectively. Figure 4-5 shows that EffVSS was increased with the decrease of temperatures and HRTs. But the increase of EffVSS was not significant enough to cause reactor failure. The EffVSS was about 67 to 75 mg/L at 5 °C in comparison to 44 to 60 mg/L at 20 °C at all HRTs. This indicated that ASBR has inherent characteristics (high F/M to low F/M) for retaining biomass within the reactor.

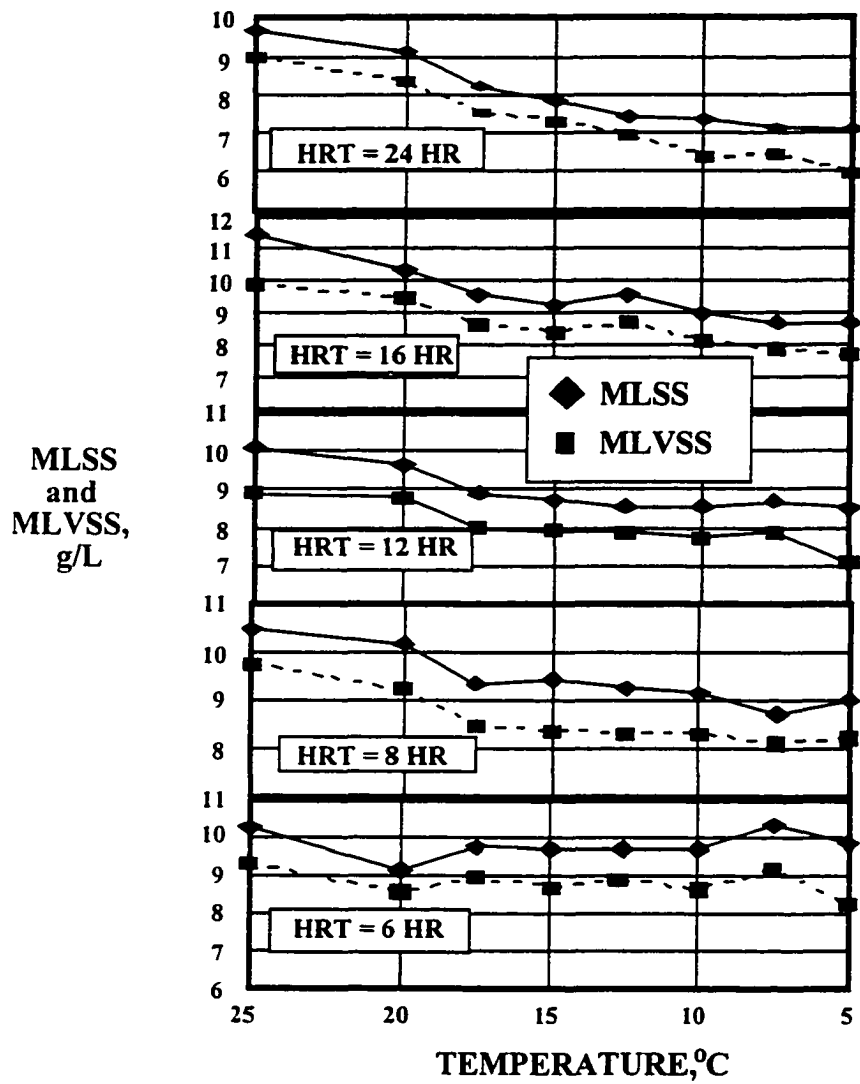


Fig. 4-4. Biomass levels at various temperatures and HRTs.

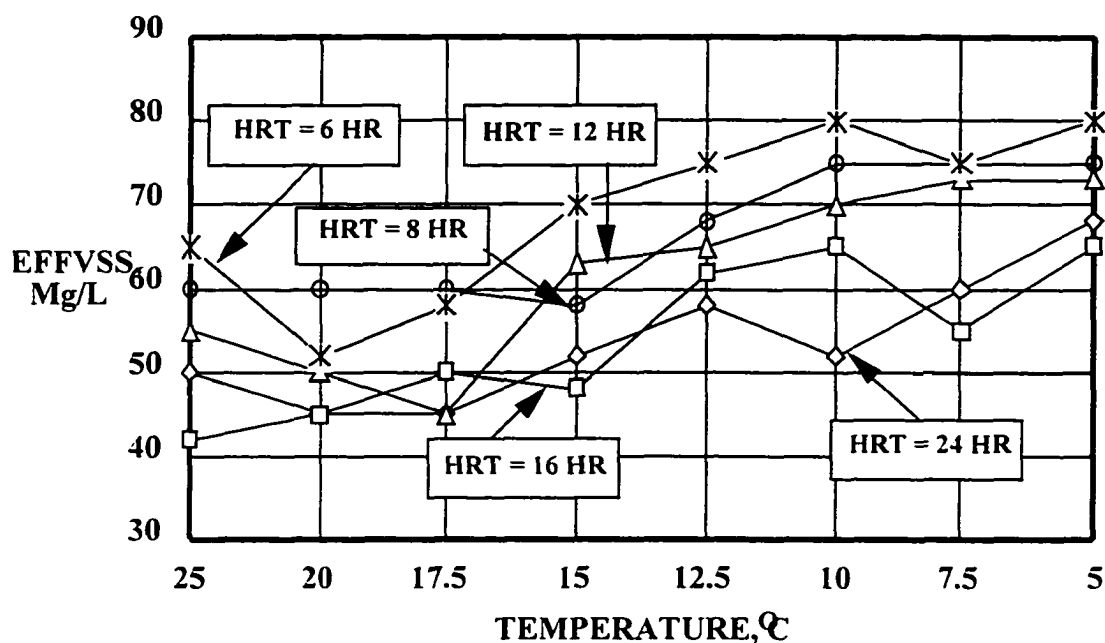


Fig. 4-5. EFFVSS at various temperatures and HRTs

The SRT decreased with the decrease in temperature and HRT (Figure 4-6). The SRT at 25 °C and at 24, 16, 12, 8 and 6 hr HRT were about 180, 152, 80, 57 and 32 days respectively. But at 5 °C, SRTs were about 89, 73, 51, 39 and 25 days at respective HRTs. At higher temperatures and longer HRT, the SRTs were higher than the minimum required for stable operation, resulting in 90% stabilization. The high SRT at higher temperatures indicated that the ASBR is very stable in removing organics and retaining biomass. Lower SRTs resulted in decreased organic stabilization at low temperatures. At lower temperatures and HRTs, SRT decreased to about 25 days, but this did not result in excessive solids wash out and reactor failure. Even the 25-day SRT was sufficient to maintain good BOD₅ removals at around 75%. If the SRT can be increased by increasing the biomass concentration in the reactor and/or by decreasing the effluent suspended solids, similar organic removal as 25 °C could be achieved at the lower temperature.

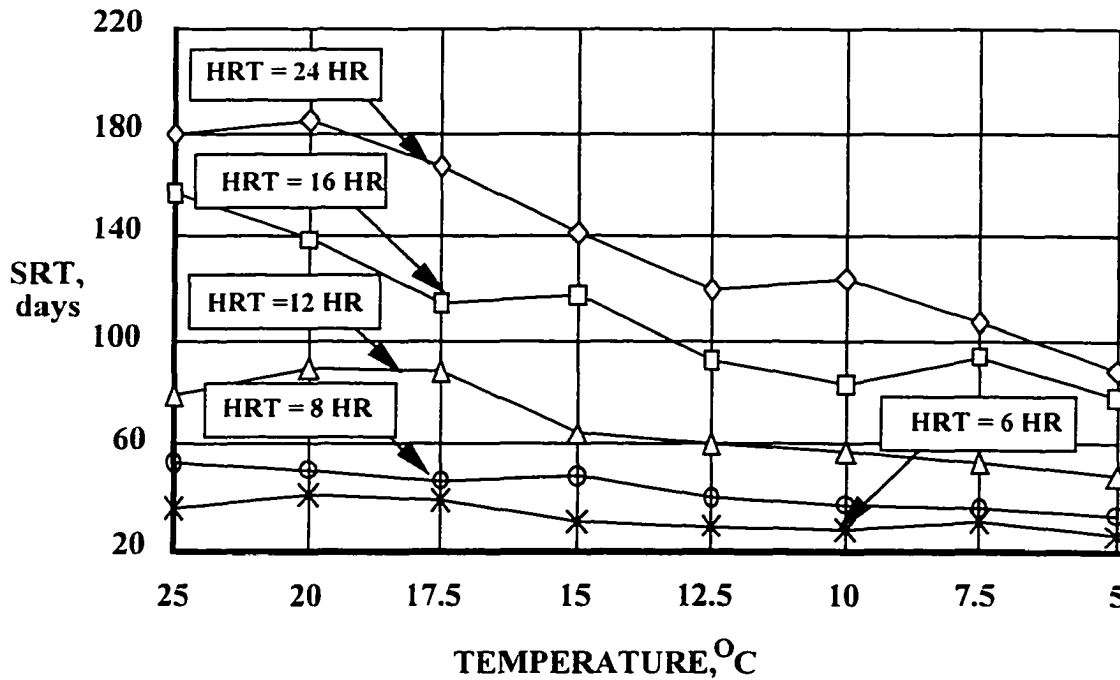


Fig. 4-6. Solid retention times (SRT) at various temperatures and HRTs

The variation in theoretical and actual methane production is illustrated in Figure 4-7. Both theoretical and actual methane production decreased with decreasing temperature, which is due to the lower organic removals occurring at the lower temperatures. At 25 °C, actual methane production increased from 1 L/d to around 3.7 L/d with the increase in COD loading from 0.6 g/L/d to 2.4 g/L/d. At 5 °C, with the same loading change, actual methane production increased from 0.58 L/d to 1.3 L/d. With the decrease in temperature and HRT, the actual methane production was much lower in comparison to theoretical methane production. This is due to the two reasons. At lower temperature, the solubility of methane in the liquid is higher, consequently more methane was lost in the liquid effluent. Another reason was due to the utilization of COD for sulfate reduction to sulfide. Sulfate reduction also varied with temperature and HRT change. At lower temperatures more sulfate reduction occurred, more COD used for sulfate reduction, resulting less methane production. This is discussed in greater detail later.

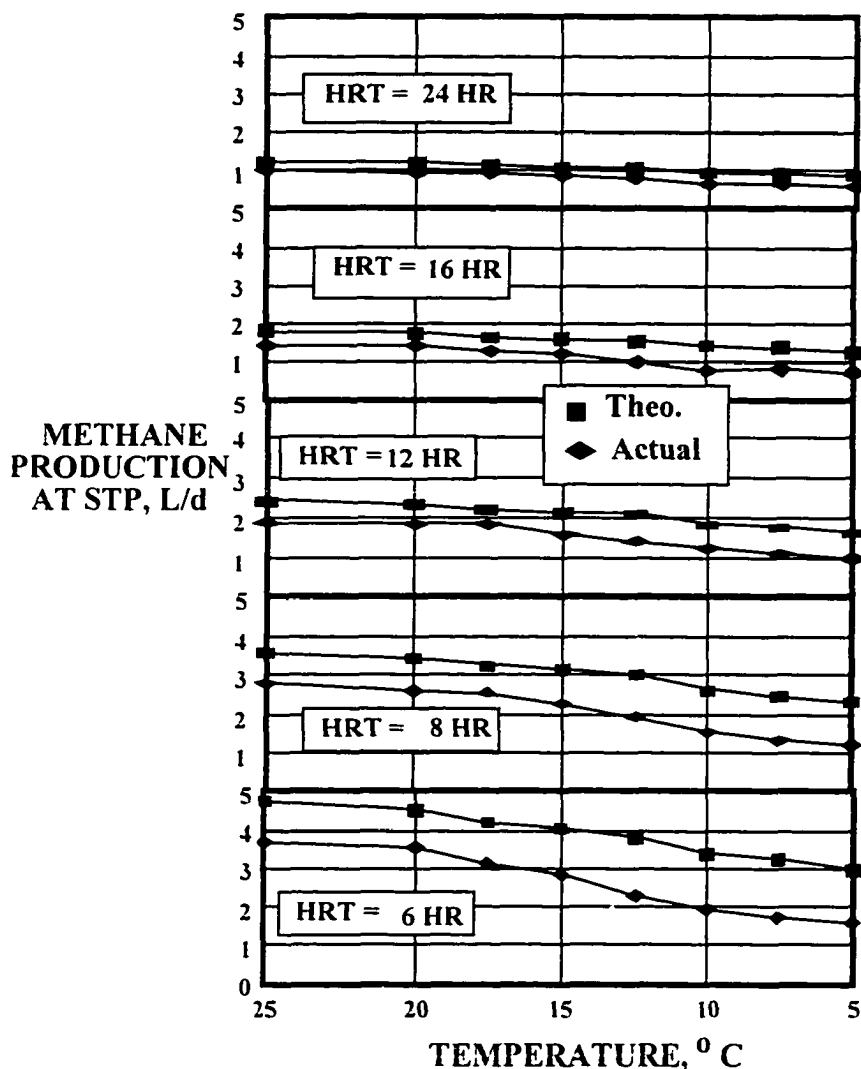


Fig. 4-7. Theoretical and actual methane production at various temperatures and HRTs.

Figure 4-8 shows that the F/M ratio is very low with a range of 0.06 to 0.30 gCOD/gMLVSS/day during decanting at all temperatures and HRTs. The F/M ratio increased with decrease in temperatures and increase in organic load. At 25 °C and 24 hour HRT, the F/M ratio was significantly lower at around 0.06 1/day, corresponding with higher organic removals. At 5 °C and a 6 hour HRT, the F/M ratio was around 0.30 1/day, corresponding to a lower organic removal and lower SRT.

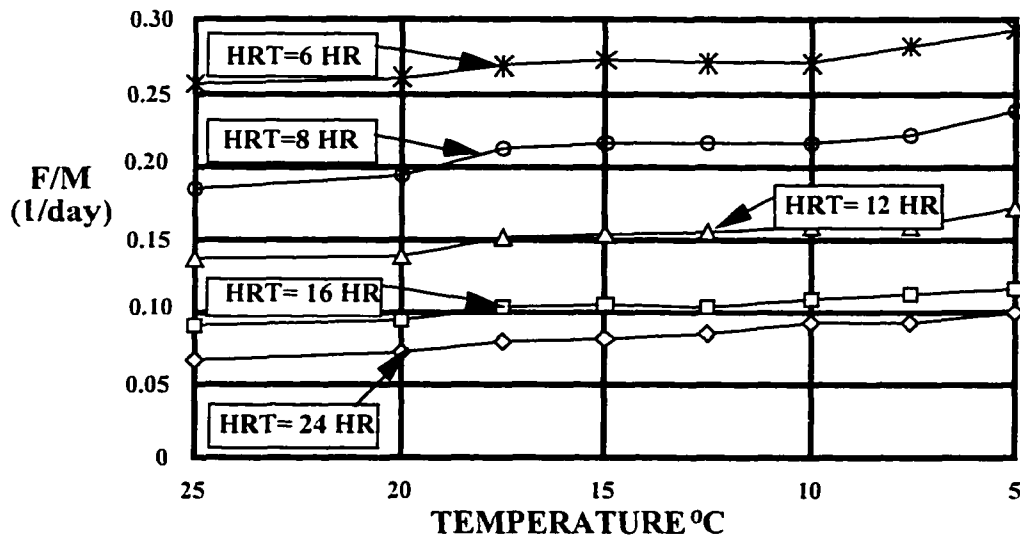


Fig. 4-8. Variation of F/M ratios at various temperatures and HRTs.

However, the maximum F/M ratio of about 0.30 1/day achieved in this research is low in comparison to a maximum F/M ratio of 1.0 1/day required for successful performances of biological system [Davis and Cornwell, 1991]. Low F/M ratios of 0.10 to 0.30 1/day shows that the systems MLVSS were generally high which resulted in good biomass separation and settling in the ASBR process.

Volatile fatty acids (VFA) concentrations in the system were very low (9 to 26 mg/L as acetate) at 25 and 20 °C and at all HRTs. The low level of VFA in the long HRT systems was attributed to high MLVSS and long SRT with the almost complete conversion of organics with little intermediaries. At the shorter HRTs of 8 and 6 hr, VFA concentration tended to be higher as 32 to 60 mg/L, particularly at lower temperatures of 10, 7.5 and 5 °C. This higher VFA at the lower temperatures and shorter HRTs are also reflected in the lower COD removal (Figure 4-2) and low methane production (Figure 4-7).

The VFA concentrations in the systems were also found to be related to the system MLVSS and SRT. The VFA concentrations were found to increase with a decrease in SRT. Similar results were reported by Dague et al. [1967]. The implication of this finding is that a

low level of biomass loss resulted in higher SRTs and lower levels of volatile acids in the reactor. The overall result was a high conversion of organics to methane. Based on the low levels of VFA and the high performance of the ASBR in this research, it can be concluded that the ASBR selected methanogens that can grow and remain active at low levels temperatures.

Ames tap water used in preparing the feed solution, and contained a reasonable amount of sulfate (95 to 130 mg/L). As from the literature review, it can be seen that presence of sulfate and sulfate reducing bacteria (SRB) has a considerable role for anaerobic treatment of dilute wastewater at psychrophilic temperatures. The data for influent sulfate, effluent sulfate, effluent sulfide, effluent sulfide in gas and sulfate reduction for COD utilization are shown in Figure 4-9. From the Figure 4-9, it has seen that sulfate reduction and effluent sulfide concentration increased with the decrease of temperature from 25 to 5 °C . At 25 °C, sulfate reduction was about 38 and 65 mg/L at HRT of 24 and 6 hr, respectively. At a temperature of 5 °C, the sulfate reduction was 103 to 115 mg/L, with little sulfate remaining in effluent (4 to 10 mg/L). This may be due to the fact that SRB become more active at low temperature and at high sulfate/COD concentration. But the effluent sulfide was about 17 to 27 mg/L (<200 mg/L) at 5 °C, depending on HRT. The low effluent sulfide in the effluent demonstrated that methanogens were still dominate with the ASBR system. The effluent sulfide in the biogas was very low or undetectable (<1%) at all conditions.

COD Balance

The performance of the anaerobic reactor system can be evaluated with in terms of organic removal and methane production. There was a direct relationship between organic removal and methane production. Methane production increased with the increase in organic removal. For all reactor systems, input must be equal to output. That means for the COD entering the reactor must be about equal to the COD leaving the reactor. As previously

mentioned, with the decrease of temperature, organic removal and methane production decreased but the sulfate reduction and effluent COD increased. So it can be hypothesized that the influent COD will be equal to sum of the effluent methane, effluent biomass, effluent liquid COD and COD utilization for sulfate reduction assuming no biomass accumulation. That is,

Influent COD = \sum (recovered methane in gas phase in terms of COD + effluent soluble COD + effluent vss COD + dissolved methane-COD in the effluent + COD used for sulfate reduction)

For example, if the influent COD is 600 mg/L, the effluent COD will be around 600 mg/L with the addition of the mentioned parameter. The dissolved methane-COD ($\text{CH}_{4\text{aq}}$ -COD) was calculated by Henry's law, which varies with the lowering temperature.

The variation of the above parameters is shown in Figure 4-9, with the change of temperatures and HRTs. Figure 4-9 can also be used to compare the different parameters with the HRT and temperatures horizontally and vertically. With the decrease of temperature (vertical movement) from 25 to 5 °C, the recovered methane decreased with the increase of COD used for sulfate reduction, $\text{CH}_{4\text{aq}}$, effluent soluble COD and effluent VSS COD. But when HRT decreased, effluent soluble COD and effluent VSS increased, decreased methane production. With the calculation of the recovered COD in the effluent, there was no substantial difference observed between influent and effluent COD.

Comparison of Performance of Different Anaerobic Systems

Table 4-1 shows comparisons of performance of various anaerobic reactors treating various substrates at different temperatures and loading conditions. Anaerobic filters performed very poorly, treating sewer waste at 5 °C and 28 hr HRT. A UASB, treating sewage at 9 to 25 °C at 2.6 to 7.2 hr HRT, removed COD in the range of 50 to 90%. The

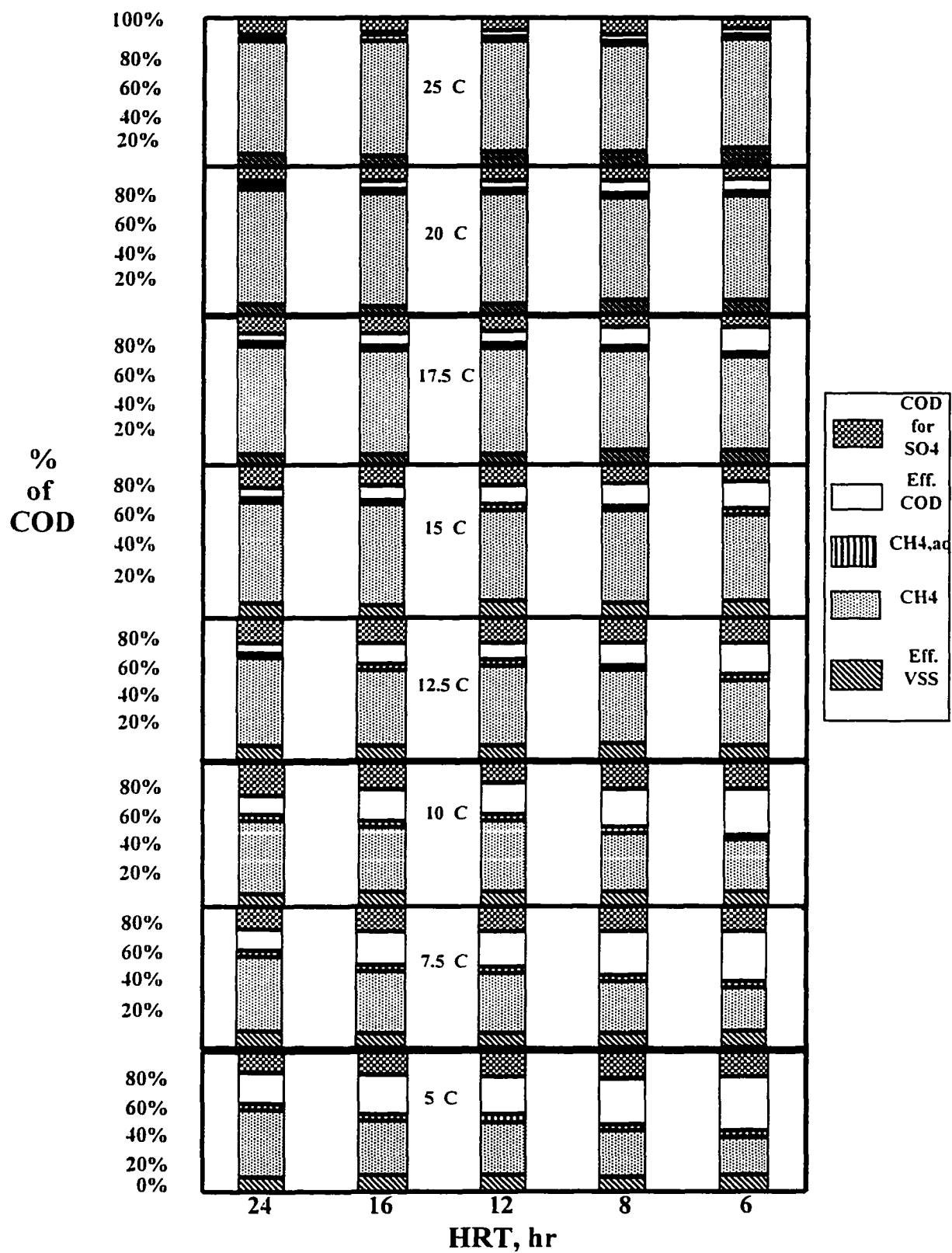


Fig. 4-9. COD Balance at various temperatures and HRTs

Table 4-1 Comparison of performances of various anaerobic treatment systems

Reactor/Waste	HRT (hr)	Temperature (°C)	waste Strength (mg/L)	Loading (g/L/d)	% Removals	References
1. Anaerobic Filter						
-Sewage	28	5	207	0.17	5	Viraraghavan et al. 1991
	57	5	286	0.11	40	
	57	10	286	0.11	47	
-Dairy Waste	24-72	12.5-30	3560	1.04-4.03	45-76	
-Sewage	5	25-33	166-515	0.8-2.5	71	Raman et al. 1978
2. UASB						
-Sewage	2.6-7.2	9-25	150-590	0.5-4.5	50-90	Sanz et al. 1989
3. AEBR						
-Synthetic	0.33-6	10,20,30	50-600	0.8-4.8	38-90	Switzenbaum et al. 1980
-Sewage	0.08-3	20	88-186	0.65-3	80	Jewel et al. 1981
4. Fluidized Bed						
-Synthetic	1, 1.5	13-31	557-700	11.2-13.4	62, 71	Maragno et al. 1992
-Sewage	2.7-26	10-25	150-590	1.0-4.5	60-90	Sauz et al. 1989
5. ASBR						
-Synthetic wastewater (NFDM)	6	5-25	600	2.4	62-95	This Research
	8	5-25	600	1.8	67-96	Do
	12	5-25	600	1.2	73-96	Do
	16	5-25	600	0.9	71-98	Do
	24	5-25	600	0.6	77-98	Do

anaerobic expanded bed reactor (AEBR) treating synthetic wastewater at temperature 10 to 30 °C and at HRT 0.33 to 6 hr, removed organics from 38 to 90%. The anaerobic fluidized bed reactors had removal efficiencies between 62 to 71% for treating synthetic wastewater at 13-31 °C and at 1-1.5 hr HRT. In the present research, the ASBR treating synthetic wastewater at temperatures ranging from 5 to 25 °C and HRT 6 hr removed soluble COD at efficiencies between 62 to 95%. The ASBR performed better, even 77-98% at 24 hr HRT at various temperatures. In comparison to other reactor systems, the ASBR performed well. This may be due to the type of substrate, long acclimation, and batch feed conditions.

CONCLUSIONS

Based on this research, it is evident that the ASBR process has intrinsic characteristics that result in a high level of organic removal, even when treating a low strength wastewater at temperatures from 25 °C to 5 °C. At 20 to 25 °C, the soluble COD and BOD₅ removals were in excess of 90%. Even at a low temperature of 5 °C and a six hour HRT, BOD₅ removal was 75%. The VFA concentration was very low (8- 60 mg/L) at various temperature and loading concentrations. This demonstrates that the ASBR can be effectively used at very low temperatures with organic loading 2.4 gCOD/L/day. The high F/M at the time of feeding and low F/M at the time of decanting played a significant role for its performance. The effluent suspended solids concentrations tended to increase with increases in organic loading and/or decreases in temperature, but the effluent solids losses were fairly compensated for by increases in biomass growth and retention. The results revealed that the application of the ASBR to the treatment of low strength, low temperature wastewaters offers the possibility of lower cost treatment of industrial and municipal wastes that are normally treated aerobically with higher expenditures of energy and increased sludge production.

CHAPTER 5

STRUCTURE AND METHANOGENIC ACTIVITY OF GRANULES FROM AN ASBR TREATING DILUTE WASTEWATER AT LOW TEMPERATURES

A paper accepted for publication in the Journal of Water Science & Technology

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ABSTRACT

The microstructure of ASBR granules treating synthetic substrate consisting of non-fat-dry-milk at temperatures of 5, 15 and 25°C was analyzed by scanning and transmission electron microscopy. The specific methanogenic activity of the granules was tested at 35°C using acetate and mixtures of acetate and propionate as substrates. Results revealed no significant variation in microbial structure for the different temperature conditions. Granules at 15 and 25°C exhibited uniform structure, predominantly *Methanothrix*-like microorganisms, while granules at 5°C indicated the existence of a layered structure. This reinforces the hypothesis that layering is largely substrate specific, but also suggests that temperature may have a role. Activity tests revealed that even after long periods of operation at psychrophilic temperatures, mesophilic bacteria are active and have the ability to rapidly degrade acetate and mixtures of acetate and propionate.

KEYWORDS

ASBR; structure; granules; psychrophilic; methanogenic; layer.

INTRODUCTION

Bacteria have the inherent capability of forming granules in natural or man-made environments. In anaerobic biological waste treatment systems granule formation and retention is one of the key concerns for environmental engineers. High-rate biological

systems can be designed to immobilize the biomass in the reactor, thus providing a solids retention time, SRT, much greater than the hydraulic retention time, HRT (Lettinga *et al.*, 1980). High-rate anaerobic systems, such as the anaerobic sequencing batch reactor, ASBR, achieve this goal by the mechanism of bacterial aggregation known as granulation (Sung and Dague, 1995). Granules result from self-immobilization of various kinds of bacteria associated with methanogenic fermentation (Bhatti *et al.*, 1993). The anaerobic bioconversion of complex organic matter to methane and carbon dioxide requires at least three functionally different trophic groups of bacteria, i.e., hydrolytic fermentative bacteria, syntrophic acetogenic bacteria, and methanogenic bacteria. The densities of the population of these bacteria in the granules varies widely depending on the chemical composition of the wastewater and bioreactor operational conditions (Bhatti *et al.*, 1993; Nishio *et al.*, 1993). The bacterial composition influences the overall characteristics of the granules and overall structure of a granule appears to be result of the response to environmental conditions (Fukuzaki *et al.*, 1991). Several mechanisms could be involved in the formation of different types of granules (Morgan *et al.*, 1991) depending on waste stream, reactor configuration, and operating conditions.

Granule formation and retention is generally thought to be the result of environmental pressure or selection, i.e., any non-granular material is washed out of the reactor (Visser *et al.*, 1991). Other mechanisms, however, have been suggested as being associated with the granulation process and subsequently with granule structure and stability. The granulation process may be initiated by bacterial adhesion to inert matter or inorganic precipitates (Dolfing, 1986; Mahony *et al.*, 1987). Bacterial agglomeration may also result from physiochemical interactions (Characklis, 1981).

It has been suggested that the filamentous bacterium *Methanothrix* plays an important role in binding granule components or nuclei together (Macleod *et al.*, 1990; Morgan *et al.*, 1991). These loosely adhered bacterial aggregates are strengthened by extracellular polymers

secreted by bacteria to form firmly attached granules (Ross, 1984; Shen *et al.*, 1993). It has also been reported that granules from a range of full-scale UASBs were affected by storage in different ways with some showing signs of integration after 6-8 weeks (Alibhai and Forster, 1986).

The start-up of ASBR systems, like other high-rate anaerobic processes, generally takes a long time. However, ASBRs seeded with mature granules require less time for start-up, compared to reactors started with flocculent seed (e.g., biomass from a conventional anaerobic digester). With the increased use of high-rate anaerobic processes, the transfer of granules for seed sludge from one treatment plant to another plant may become more common. It is therefore of great technical and commercial interest to examine the changes in granular sludges at different operational and environmental conditions. The main objectives of this study was to elucidate and compare the composition and characteristics of three kinds of granular sludges having the same origin and treating the same organic wastes in three reactors under different loading and temperature conditions. The results provide valuable information on process performance and insights into the mechanisms of granulation.

MATERIALS AND METHODS

ASBR Operation. Experiments were carried out in three laboratory-scale ASBRs each with an effective volume of 6 L. The reactors were made from 12 mm cylindrical Plexiglas. The experimental setup has been presented elsewhere (Banik and Dague, 1996). Methanogenic granules for start-up were supplied from an ongoing pilot-scale ASBR study at the City of Cedar Rapids Water Pollution Treatment Plant, Cedar Rapids, Iowa. The initial granule concentration was approximately 12 g/L MLSS, with a volatile fraction of approximately 90%.

Feed to the bioreactors was a synthetic waste, non-fat-dry milk (NFDM). The composition of NFDM was approximately 51% lactose, >36 % protein, fat <1% and 8.2%

ash (Banik and Dague, 1996). Sodium bicarbonate was added as a buffer, and trace minerals (Fe, Zn, Ni, Co, Mo) were added to provide adequate nutrients for granulation. Reactor operation started with a COD substrate concentration of 1000 mg/L at 25°C and a HRT of 24 h. After obtaining near steady-state conditions (consistent gas production, gas composition, and COD removal efficiency), the substrate concentration was changed from 1000 mg/L to 600 mg/L. The reactors were fed a COD concentration of 600 mg/L throughout the remainder of the study. Each bioreactor was operated at two HRTs at each of the following temperatures: 25, 20, 17.5, 15, 12.5, 10, 7.5, 5°C. The three reactors were operated at HRTs of 24 and 12 h, 16 and 8 h, and 12 and 6 h, respectively. Additional details on start-up and operation have been published previously (Banik and Dague, 1996).

Methanogenic Activity Test. The methanogenic activity tests were carried out at 35°C in duplicate for granules grown at temperatures of 25, 15, 10 and 5°C, using 250 mL bottle contain the batch medium consisting of acetate or a mixture of acetate and propionate and granules. The amount of granules was sufficient to ensure that a measurable amount of methane would be produced within 2 to 4 h. The methanogenic activity test procedure followed that of Rinzema, *et al.* (1988).

Electron Microscopy. Sample preparation for scanning electron microscopy (SEM) consisted of placing granules in sealed 50-mL serum bottles which contained 4% glutaraldehyde in anaerobic 0.05 M cacodylate buffer. Fixation was carried out overnight at 4°C. The fixed granules were washed with buffer three times, and again fixed with 1% osmium tetroxide. The whole granules were dehydrated with a graded series of ethanol in distilled water from 50 to 100% (v/v). The specimens were critical point dried in CO₂, mounted on brass discs, and sputter coated in a Polaron E5100 with platinum/palladium target (60:40). The prepared specimens were examined with a JEOL JSM-35 SEM at an accelerating voltage of 20 kV. Examinations of bisected granules under SEM illustrated the

morphology and location of individual bacteria inside the granule.

Sample preparation for transmission electron microscopy (TEM) consisted of embedding the ethanol dehydrated specimen (as prepared for SEM) in epoxy resin and curing for 24 h at 60°C. The cured specimen was cut with a diamond knife on a Reichert Ultracut E ultramicrotome and placed on copper grids. Thin sections were post-stained with uranyl acetate and lead citrate and photographed on a JEOL 1200EX at an accelerating voltage of 80 kV. The SEM and TEM analyses were conducted according to the procedure described previously by Horner *and Wagner*, (1980). TEM examination illustrated species diversity and community structure.

RESULTS

The size of the granules grown at 5°C was approximately 1.8 - 2.8 mm, with a densely packed outer layer (Figure 5-1a). As seen in Figure 1b, the granule surface had a complex bacterial composition with some cavities, possibly resulting from gas production. The methanogens in the outer layer were of diverse morphologies, including cocci, bacilli, and some filaments (Figure 5-1d). The inside of the bisected granules was densely packed with bacteria of various morphologies (Figure 5-1e), but the bisected granules had macropores which likely alleviated mass transfer limitations inside the granules (Figure 5-1c). The center core of the granules was loosely packed with two types of cell structure typical of *Methanothrix* (Figure 5-1f). The outer layer consisted of a uniform and less dense bacterial consortium (Figure 5-1g).

The typical size of the granules grown at 25°C was approximately 1.5-2.5 mm. These granules did not exhibit a layered structure (Figures 5-2b-f). SEM of the outer layer illustrated that the granules comprised of predominantly of cocci and some bacilli and filaments (Figure 5-2b). The bisected granule indicated a complex composition. Both the bisected midsection and outer layer were comprised of predominantly *Methanothrix*-like

cells. Analysis by TEM illustrated that the granules did not have a wide variety of species and were predominantly composed of loosely packed *Methanothrix*-like cells, inorganic material, and extracellular polymers (Figures 5-2f and 2g).

Figure 5-3 illustrates the SEM for granules grown at 15°C at HRTs of 16 and 8 h. The granules were irregular in shape at both HRTs and the granules grown at the higher loading rate were slightly larger (Figure 5-3a and 3b). The granule surface was comprised of cocci, bacilli and some filaments at the 8 h HRT (Figure 5-3d). The surface for granules at the 16 h HRT was complex, with loosely packed bacteria and inorganic materials. The outer layer consisted of microorganisms typical of *Methanothrix* at both HRTs and the higher loading resulted in a more diverse community structure. There was no significant difference between granule interior and exterior (data not shown).

Table 5-1. Specific methanogenic activities of ASBR granules at different temperatures, g COD/g VSS d.

Temperature, °C	Substrate:	Acetate	Acetate and Propionate (1:1 ratio)			
	IIRT, h					
	24	16	12	24	16	12
5	1.62	1.75	1.78			
10	1.74	2.08	2.13	0.41	0.46	0.48
15	1.82	2.19	2.22	0.52	0.53	0.58
25	1.91	2.37	2.42	0.71	0.72	0.77

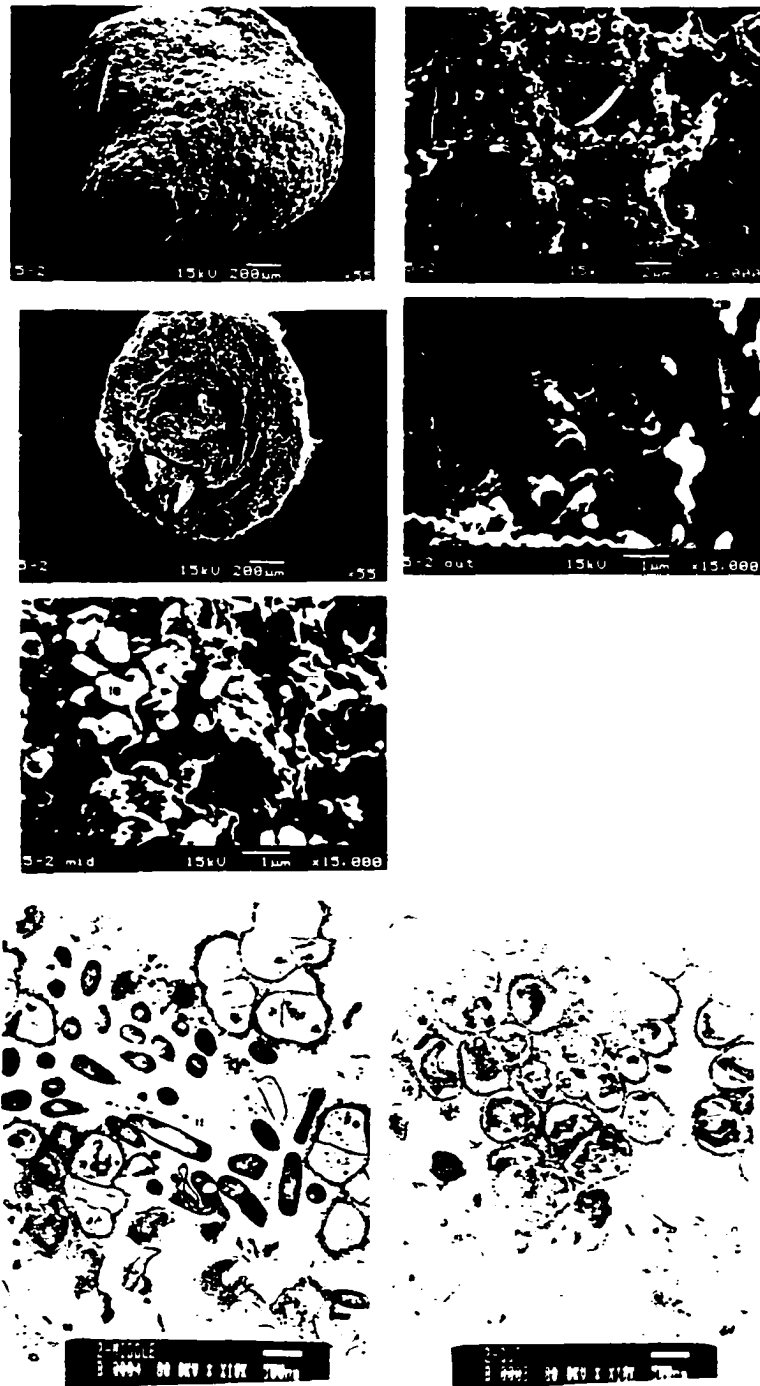


Fig. 5-1. Scanning and transmission electron micrographs of granules from an ASBR operating at 5°C. From top left: 1a and b. SEM of granule surface. 1c. SEM of bisected granule. 1d. SEM of bisected granule near granule exterior. 1e. SEM of bisected granule near center of granule. 1f. TEM of thin section near granule center. 1g. TEM of thin section near granule exterior.

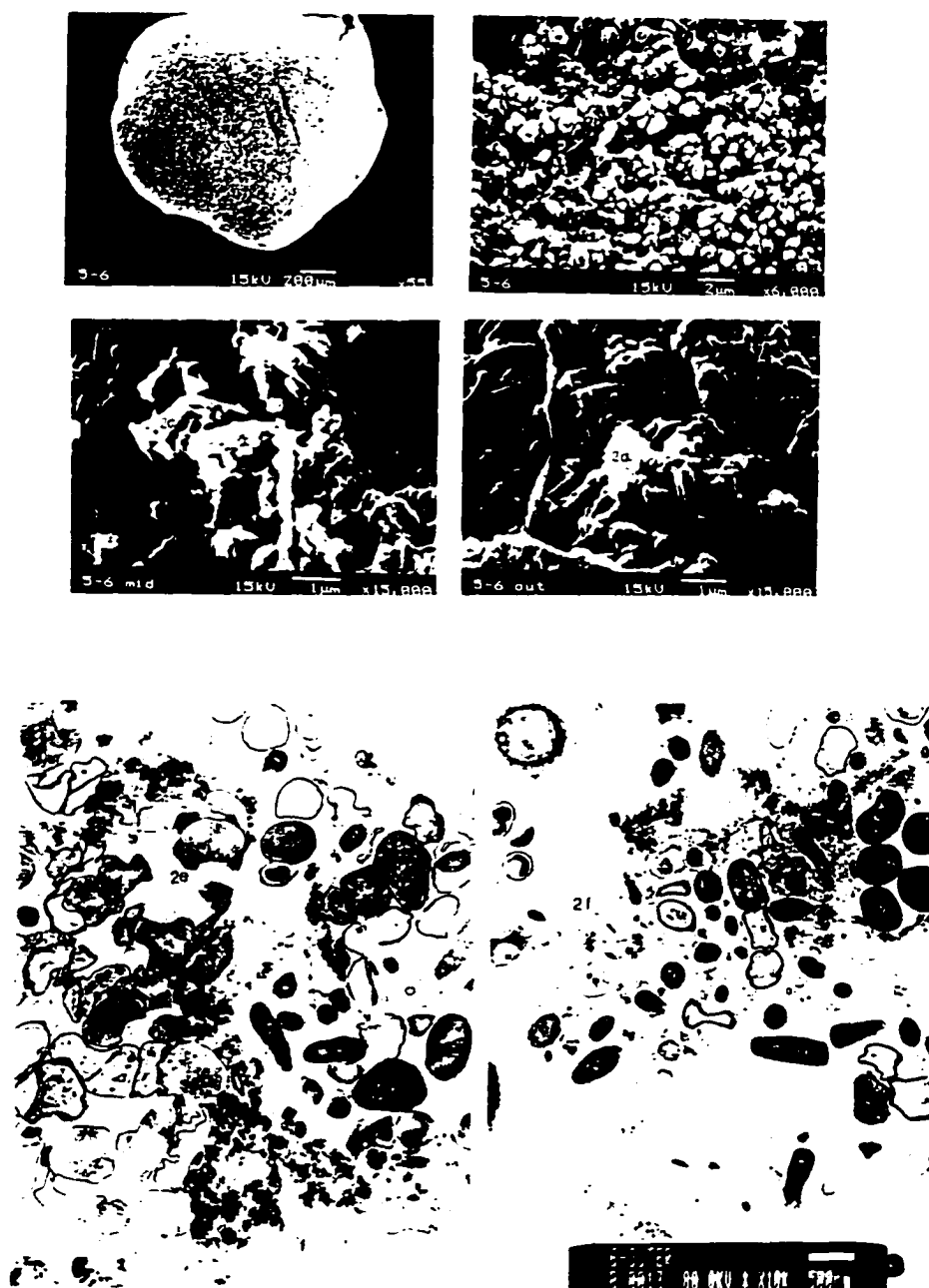


Fig. 5-2. Scanning and transmission electron micrographs of granules from an ASBR operating at 25°C. From top left: 2a. and b. SEM of granule surface. 2c. SEM of bisected granule near granule center. 2d. SEM of bisected granule near granule exterior. 2e. TEM of thin section near granule center. 2f. TEM of thin section near granule exterior.

From Table 5-1 it is evident that the specific methanogenic activity of granules at all temperatures increased as the HRT decreased. The activity of granules grown at psychrophilic temperatures (5, 10, and 15°C) was slightly lower than the activity of granules grown at mesophilic temperatures (25°C). The activity of granules using both acetate and propionate was much lower than with acetate alone, indicating a lower maximum specific growth rate for propionate. Guiot *et al.* (1988) measured activities ranging from 1.3 to 2.6 g COD/g VSS d for granules grown on sucrose at 27-29°C. Whereas Wiegant (1986) reported activities up to 7.3 g COD/g VSS d for thermophilic granules (55°C) cultivated on acetate and butyrate. It is expected that the activity of granules will be lower when they are grown on actual wastewaters due to the presence of slowly biodegradable constituents.

DISCUSSION

Only a limited number of studies have reported on the microstructure of anaerobic granules, and most of these report on granules from UASB reactors. Grotenhuis *et al.* (1991) reported that there was no layered structure for UASB granules treating propionate, ethanol, and sugar-refinery wastewaters. In propionate degrading granules, *Methanothrix* and *Methanobrevibacter arboriphilus* were found clustered together with the propionate-oxidizing acetogens throughout the granules. Macleod, *et al.* (1990) examined granules degrading sucrose in an UASB and proposed a three layered granule structure. The outer and middle layers were composed of mainly acidogenic and syntrophic microcolonies, respectively, while the center core was composed of *Methanothrix*-like methanogens. Guiot and Costerton, (1992) confirmed the existence of layered structure with granules grown on glucose. Fang *et al.* (1994) observed a layered structure from sucrose and brewery degrading granules, but not for granules degrading glutamate. These observations suggest that the bacteriological composition and microstructure of granules depend largely on the growth substrate.

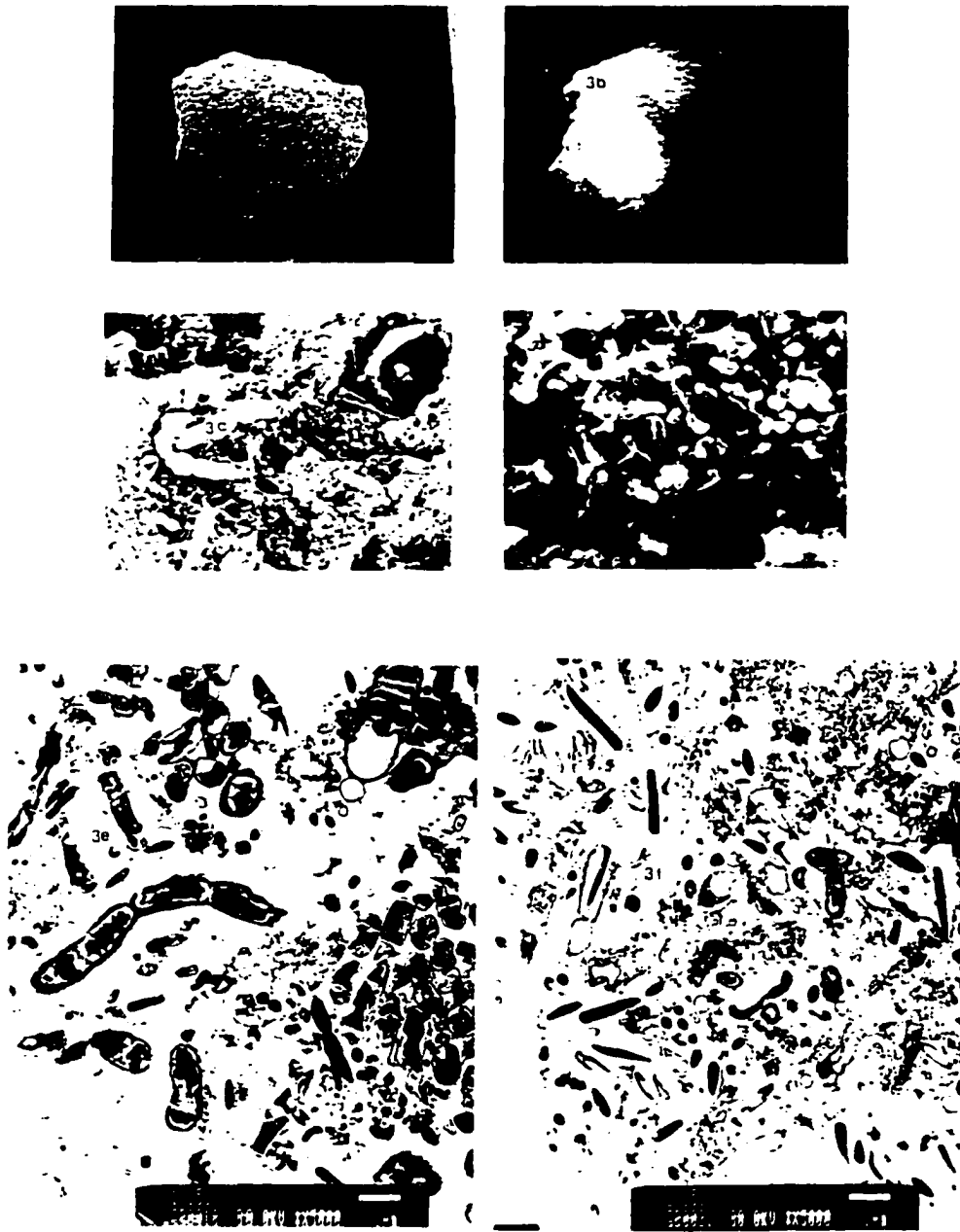


Fig. 5-3. Scanning and transmission electron micrographs of granules from an ASBR operating at 15°C at different loadings. From top left: 3a. and b. SEM (magnification 54X) of granule surface for 16 and 8 h HRT, respectively. 3c. and d. SEM (magnification 6,000X) of granule surface for 16 and 8 h HRT, respectively. 3e. and f. TEM (magnification 5,000X) of bisected granule for 16 and 8 h HRT, respectively.

It is possible that granule bacterial microstructure and profile depends on the concentration profile of substrate and metabolites, such as hydrogen, VFAs, acetate, etc. These profiles are dependent on the rate of biodegradation and diffusion into the granule. In this study, ASBR granules treating non-fat-dry milk at temperatures of 15 and 25°C did not exhibit a layered structure. One possibility is that the complex nature and dilute concentration of the feed precluded competition for attachment at the granule surface. This may be due to the high protein content of the feed (>36%). In this study, the slowly degraded non-fat-dry-milk may have been evenly distributed throughout the granule. This would have prevented a significant concentration gradient of metabolites such as VFAs, acetate, hydrogen, etc. into the granule interior. Consequently, the bacteria were rather evenly distributed throughout the granules.

As the temperature shifted from mesophilic (25°C) to psychrophilic (5°C), there was a slight shift in the microstructure of granules. The granules grown at 5°C had more diversity of species on the inside of the granules than on the exterior, possibly indicating the existence of a layered structure. The overall performance of the reactor with respect to organic removals was less at psychrophilic temperatures (75% BOD₅ removal at 5°C and 6 h HRT versus >90% BOD₅ removal at 25°C, Banik and Dague, 1996). The specific methanogenic activity of granules grown at the lower temperatures was also lower.

The relatively high specific methanogenic activity at 35°C using granules grown at 5 and 10°C indicates the existence of mesophilic microorganisms and/or the existence of psychrophilic microorganisms with the ability to grow at mesophilic temperatures. The granule structure observed at 5°C was noticeably different (more layered structure) than the structure at 15 and 25°C, but the existence of obligate psychrophilic microorganisms was not confirmed. From these results it can be concluded that the cultivation of obligate psychrophilic microorganisms was apparently not a prerequisite for successful anaerobic treatment at low temperatures.

CONCLUSIONS

Psychrophilic temperatures did not have a dramatic effect on the microstructure of the ASBR granules, with the possible exception of encouraging a layered structure at the lower (5°C) temperature. Granules grown on non-fat-dry-milk at 15 and 25°C exhibited a rather uniform structure, demonstrating the absence of a layered structure. This confirms the hypothesis that granule microstructure depends to a large extent on the nature of the substrate. The variation in microstructure of granules grown at 5°C, however, suggests that temperature also plays an integral role in granule composition. The granules at all temperature consisted of predominantly *Methanothrix*-like microorganisms as the key structural element, which seems to suggest that *Methanothrix* plays an important role in granulation and retention in high-rate anaerobic processes such as the ASBR. The specific methanogenic activity (SMA) of granules grown at psychrophilic temperatures were slightly less than that of granules grown at mesophilic temperatures. The production of methane at 35°C from granules grown at psychrophilic temperatures indicated the existence of mesophilic microorganisms and/or psychrophilic microorganism that could grow at mesophilic temperatures. The existence of obligate psychrophilic microorganisms was not confirmed. The specific methanogenic activity for granules biodegrading mixtures of acetate and propionate was significantly lower than that for acetate alone.

CHAPTER 6**GRANULE SIZE DISTRIBUTION OF AN ASBR OPERATED AT PSYCHROPHILIC TEMPERATURES**

A paper to be submitted to the Journal of American Filtration and Separation

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ABSTRACT

The effect of psychrophilic temperatures on methanogenic granule size distribution was evaluated in three laboratory-scale ASBRs, fed with nonfat dry milk as the sole carbon and energy source. The granules were collected at temperatures of 25, 20, 15, 10 and 5 °C and at hydraulic retention times (HRTs) of 24, 16 and 12 hours during pseudo steady-state conditions. Granule size distribution, as mean, area-weighted mean and geometric mean, were determined by automated image analysis (AIA).

Results showed that psychrophilic temperatures appear to have an effect on the granule size distribution. The mean size of the granules increased as temperatures increased from 25 to 15 °C then decreased with a further reduction in temperature. The equivalent diameter of granules was 2.0-3.3 mm for all temperatures and HRTs, with a low percentages of fines in the biomass at these temperatures and HRTs. A low percentage of fines in the reactor indicated that the ASBR selected granules, and newly formed fines are removed with the liquid effluent. No correlation exists between granule ash content and either temperature or HRT, but a positive correlation exist between granule density and granule ash content. The proposed size distribution model accurately described the size distribution of ASBR granules at various low temperatures.

KEY WORDS

Granule, ASBR, Size distribution, Psychrophilic temperatures, HRT

INTRODUCTION

Successful operation of anaerobic reactors depends on the accumulation and retention of methanogenic biomass which has high settling velocity and high methanogenic activity (Lettinga et al. 1987). Microbial granulation results from self-immobilization of various kinds of bacteria associated with methanogenic fermentation. The anaerobic bioconversion of complex organic matter to methane and carbon-dioxide requires at least three trophic groups of bacteria, i.e., hydrolytic fermentative bacteria, syntrophic acetogenic bacteria, and methanogenic bacteria. The densities and formation of these bacteria in the form of granules vary widely, depending on the chemical composition of wastewater and operational conditions (Bhatti et al. 1993).

The granulation process may be initiated by bacterial adhesion to inert matter, inorganic precipitates (Lettinga et al. 1980; Mahoney et al. 1987), and /or adhesion to each other mainly through physicochemical interactions (Charakils, 1981). These substances serve as initial carriers (nuclei) for a new bacterial growth. These loosely adhered bacteria aggregates are strengthened by extracellular polymers secreted by bacteria to form firmly attached initial granules (Allison and Sutherland, 1987). These initial granules grow larger and larger, and finally become mature granules, if favorable environmental and operating conditions are maintained.

Low temperatures influence the performance of biological system by physio-chemically and structurally changing the bacteria in the granules (Banik et al. 1997a). Well developed granules are characterized by high stability, high settleability and high methanogenic activity, which permit use of a relatively small anaerobic systems to provide efficient treatment of large wastewater streams. Settleability is one of the key parameters of granules influencing the retention of biomass in the reactor. Settleability is dependent on the density and size of the granules. Density of granules is influenced by the presence of inorganic precipitates and the inclusion of gas (Hulshoff Pol et al. 1986). The average size of

the granules is dependent on interactions of multiple factors, including temperature, substrate limitation inside the granules, granules growth rate, type and amount of nutrient supply, formation of inorganic precipitates and gas production. Low temperatures influence the accumulation and retention of biomass by increasing liquid viscosity, and by lowering biomass growth rates (Banik et al. 1997b). Particle size distribution has been considered here as a characteristic of granulated biomass. In this study, the effect of low temperatures and hydraulic retention times (HRT) on particle size distribution was evaluated. A size distribution model was also developed which describes the size distribution in ASBR system.

MATERIALS AND METHOD

ASBR Operation: This study was carried-out in three laboratory scale ASBRs, each with an effective volume of 6 L. The experimental setup has been presented elsewhere (Banik et al. 1996a). Methanogenic granules for start-up were supplied from an ongoing pilot-scale ASBR study at the city of Cedar Rapids Water Pollution Treatment Plant, Iowa. A synthetic substrate, nonfat dry milk (NFDM), was fed to the reactors. The composition of NFDM was approximately 51% lactose, >36% protein, <1% fat, and 8.2% ash. Sodium bicarbonate was added as a buffer, and trace minerals (Fe, Zn, Ni, Co, Mo) were added to provide adequate nutrients to the granules. Reactor operation was started with a COD substrate concentration of 1000 mg/L at 25 °C and an HRT of 24 hr. After obtaining pseudo steady-state conditions (consistent gas production, gas composition, and COD removal efficiency), the substrate concentration was changed from 1000 mg/L to 600 mg/L. Each reactor were operated at two HRTs at temperatures of 25, 20, 17.5, 15, 12.5, 10, 7.5 and 5 °C. The three reactors were operated at HRTs of 24 and 12 h, 16 and 8 h, and 12 and 6 h, respectively. The substrate concentration of 600 mg/L remain constant throughout the study.

Biomass Sampling: The reactor biomass was collected from three samples ports located at several heights. Mixing was initiated during collection to insure a representative sample. The height of the ports from the bottom of the reactors were at 8, 12 and 16 inches, respectively. The biomass was collected at each temperature and HRT during steady-state conditions, but the result presented here are for HRTs 24, 16 and 12 hours and temperatures of 25, 20, 15, 10 and 5 °C. The granule distribution before start-up and upon completion of operation was also evaluated.

Physical Characteristics of Biomass: The granule size distribution was determined by Automated Image Analysis (AIA). Images were collected using an RS-170 video camera attached to a Olympus stereo microscope. Approximately 25 particles suspended in water were placed in specially prepared slides. The slides consisted of an open well 25 mm in diameter and 2 mm deep. Distilled water was used to fill the well to level to avoid stray reflections from a meniscus either above or below the top of the well. Images were digitized at 640x480 pixels and stored in TIFF format for later analysis. Six frames collected per sample for a total of approximately 150 particles per sample. Images were analyzed using Noesis Vision's Visilog image analysis software (version 4.1.5). A global threshold was used to pick the dark globules from the light background. Some manual editing of the binary image was necessary to separate contacting particles. The area and perimeter were measured for each particle. A variety of measurements were then calculated from these basic measurements including area-equivalent diameter (the diameter of a circle with the same area). Size distributions were calculated showing particle count, area, and volume fractions as a function of size using both logarithmic and linear scales. Ordinary mean diameter, area-weighted mean diameter (i.e., $\text{Sum}(d^3)/\text{Sum}(d^2)$), and geometric mean diameter were calculated. Geometric mean diameter were calculated on the basis of the following formula:

$$d_g = (d_1 * d_2 * \dots * d_n)^{1/n}$$

Suspended solid (SS), Volatile suspended solid (VSS) and ash content were determined according to Standard Methods (1995). Ash content was determined as the difference between SS and VSS. VSS density was determined according to the method described elsewhere (Satoshi et al. 1995).

RESULTS

The detailed data for reactor performance and biomass concentration at different temperatures and HRTs can be seen in previous papers (Banik and Dague, 1996a, 1996b). The reactor performance decreased with the decrease of both temperature and HRT. The granule size distribution in respect to mean diameter, area-weighted mean diameter and geometric mean diameter at temperatures of 5, 10, 15, 20 and 25 °C is presented in Table 6-1. From the table it is evident that granule mean size diameter increased from start-up (25 °C) to 15 °C for all three reactors. But the mean diameter size decreased as temperature decreased from 15 C to 5 °C.

The granule mean size diameters at the shut down conditions (25 °C) were higher than at the start-up conditions, which indicated the overall growth of granules inside the reactor due to the long time operation of the ASBR.

Area-weighted mean size distribution followed the same trend as mean size distribution. The size increased from start-up (25 °C) to 15°C and after then decreased with lowering temperature at all HRTs. The area weighted mean size distributions are higher than the mean diameter at all temperatures and HRTs. The size range was 2.15-2.47, 2.12-2.76 and 1.95-2.75 mm for 24, 16 and 12 hour HRTs, respectively.

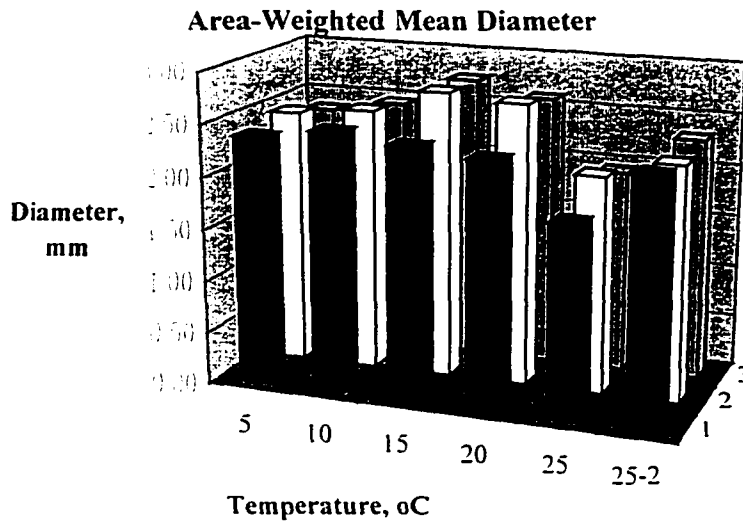


Fig. 6-1. Area-weighted mean diameter at various temperatures and HRTs.

Table 6-1. Characteristics of ASBR Granules

Temperature, °C	5	10	15	20	25 Start-up	25 Shut Down
HRT = 24 hours(R-1)						
Mean Dia., mm	1.88	1.86	2.12	2.02	1.68	1.75
Area-weight Dia., mm	2.37	2.47	2.39	2.33	1.82	2.32
Geometric Dia., mm	1.68	1.52	1.53	1.91	1.5	1.47
VSS, g	6.02	6.44	7.33	8.34	9.33	
VSS Density, g/l	1045	1006	1012	1035	1025	
Ash, %	9.50	6.08	6.63	8.80	7.25	
HRT = 16 hours(R-2)						
Mean Dia., mm	1.96	2.00	2.33	2.23	1.72	1.62
Area-weight Dia., mm	2.36	2.53	2.76	2.7	2.12	2.25
Geometric Dia., mm	1.75	1.70	1.77	2.09	1.37	1.58
VSS, g	7.65	8.16	7.95	9.41	9.86	
VSS Density, g/l	1026	1030	1029	1022	1068	
Ash, %	8.30	8.80	8.90	8.20	14.12	
HRT= 12 hours(R-3)						
Mean Dia., mm	1.87	2.05	2.19	2.12	1.60	1.91
Area-weight Dia., mm	2.35	2.46	2.75	2.61	1.95	2.25
Geometric Dia., mm	1.64	1.84	2.00	1.86	1.47	1.74
VSS, g	7.08	7.78	7.87	9.66	8.89	
VSS Density, g/l	1030	1042	1051	1040	1062	
Ash, %	9.10	9.70	11.10	9.50	13.10	

The granule geometric mean size diameters also showed an increasing trend from start-up to 20 °C at 24 and 16 hour HRTs, and from start-up to 15 °C at a 12 hour HRT. The geometric mean sizes are lower than both of the mean size and area weighted size distributions at all conditions. The geometric mean size distributions were 1.47-1.91, 1.37-2.09, 1.47-2.0 mm at 24, 16 and 12 hour HRTs, respectively.

For an HRT of 16 hours, the mean size, area weight mean size and geometric mean size were higher than those of both a 24 and 12 hour HRT. The smaller size at a 24 h HRT may be due to the substrate limitations inside the granules. The small size for a 12 h HRT, may be due to the shear stress due to higher gas production for increased organic loads.

Figure 6-2 demonstrates the individual size distribution at each temperature at an HRT of 24, 16 and 12 hours on the basis of count fraction and area fraction basis. From the count fraction basis, it is evident that about 60-80% of the particles belonged to the 2.0 to 3.2 mm equivalent size diameter range. Few particles (about 20-30 %) existed below 1.5 mm and above 3.2 mm. This indicates that the ASBR selected and accumulated larger granules. However, on the basis of area weighted basis, most of the granules (45-65 %) were about 3 mm equivalent diameter size. Very few particles (10 %) were below 1.5 mm equivalent diameter size. About 10-30 % of the particles were above 3 mm equivalent size. In this study, the small percentages of fines both on count and area weight basis may be due to the low influent substrate concentration (COD = 600 mg/L) and removal of fines from the reactor via the liquid effluent.

The VSS density and ash percentages are also shown in Table 6-1. The ash content of the granules did vary over the temperature range of 5-25 °C. The ash content was 6-15 % for all temperatures and HRTs, which indicated that the ASBR can retain highly active granules. The VSS density of the granules varied from 1006-1068 g/L. There is no clear relation exist between ash content and temperature and HRT, but a strong correlation was found between

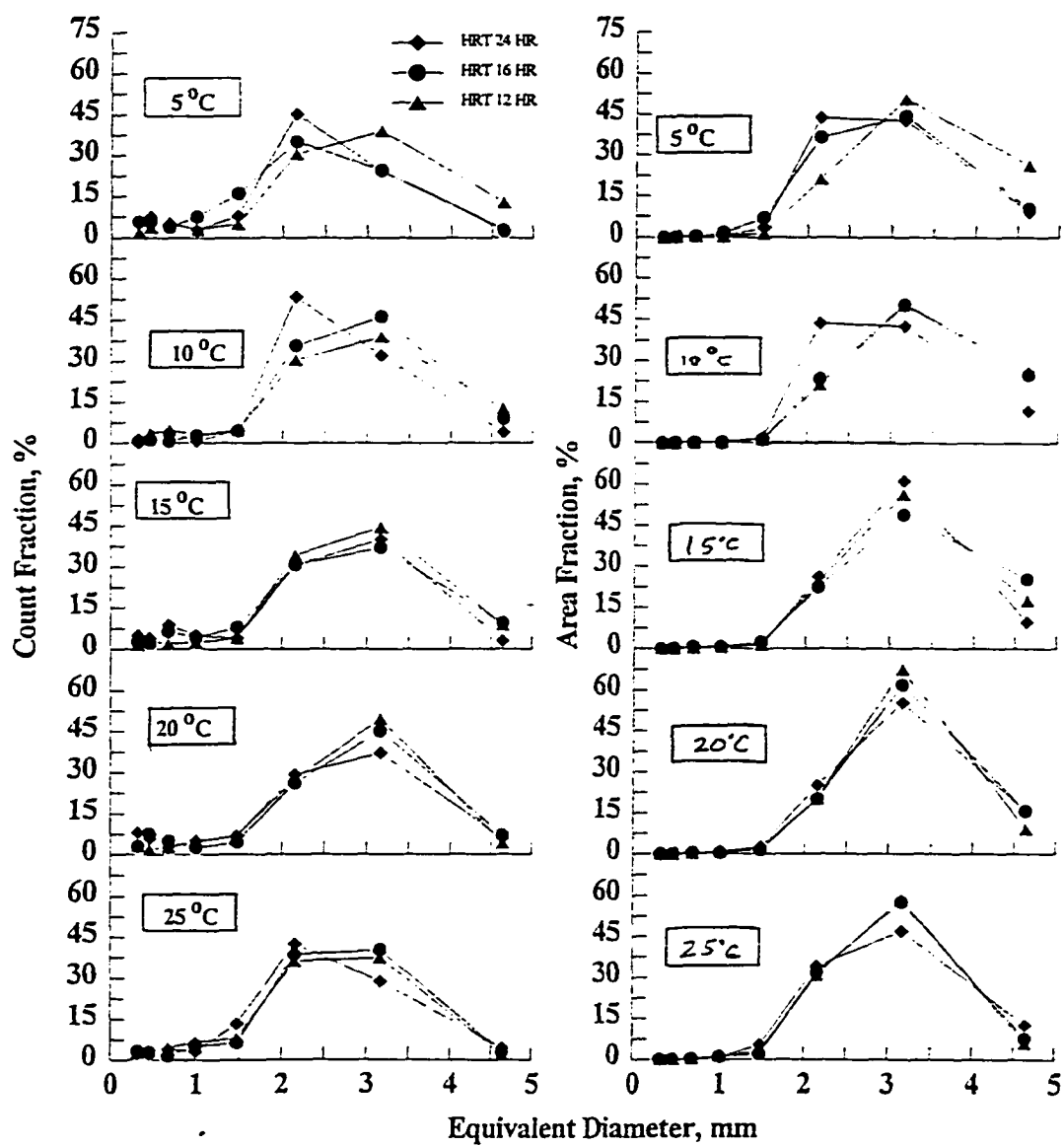


Fig. 6-2. Equivalent granule size distribution at each temperature and HRTs on the basis of count and area fraction basis.

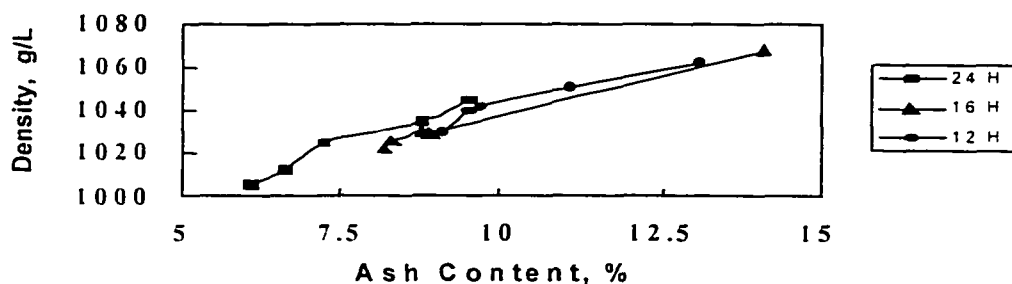


Fig. 6-3. Variation of granule density with granule ash content.

the ash content and VSS density, which can be seen in the Figure 6-3. This Figure shows that density increased with increased ash content.

DISCUSSION

Granule size distribution was used as a characteristic parameter to determine the effect of temperature on granules. Results show that the particle size of methanogenic granules depends on the reactor temperature, although the variation is small. Larger granules were obtained in the higher range of psychrophilic temperatures (15-25 °C). At lower temperature, due to the higher viscosity of liquid, more biomass was lost with the liquid effluent, resulting in lower biomass mean size (5-15 °C).

Area weighted image analysis has several advantages for the direct measurement of the surface of each granule, over measurement of mean and geometric mean diameter. The area weighted mean diameter at all temperatures and HRTs was constant, revealing that there is no significant variation of size distribution.

The increase/decrease in mean granule size in the experiments reported here partly be explained by growth, substrate depletion and decay of biomass. Two opposite force such as substrate depletion at the center of the granules and acclimation play the vital role behind the

increase of granule size increase at temperature range 25-15 °C, and decrease at 15-5 °C. At higher ranges of psychrophilic temperatures, the liquid is less viscous, diffusion limitations are less, which results in more substrate utilization and more biomass growth. With the long time operation, the granule became better acclimated to reactor conditions, resulting in more growth and higher biomass size. Acclimation of granules inside the reactor is one of the reason for increasing granule size as temperature decrease from 25 to 15 °C. Acclimation rather than substrate depletion may be the dominant force in the temperature range of 25-15 °C for increasing the biomass size. In Figure 6-4a, granules become larger and larger, and little substrate depletion occur with time. The dark color inside the granules indicated substrate depletion. This increase in substrate utilization in the core of granules leads to increased granule size, and forming newer fine granules. At lower ranges of psychrophilic temperatures (Figure 6-4b), due to the more viscous of liquid, substrate depletion does occur in the granule core which results in less substrate utilization, low biomass growth and a decrease in granule size. Although acclimation is more in this range, but due to the substrate depletion inside the granules biomass size decrease. The granule loss in the liquid effluent consisted mainly of fines, which are freshly formed from the granulation process.

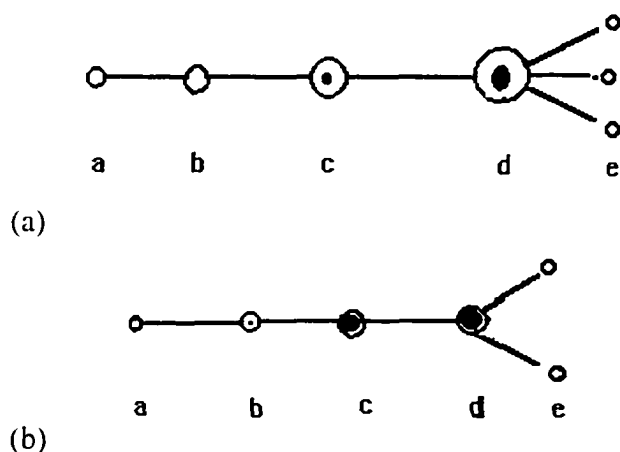


Fig. 6- 4. Growth of Granules with the time. Black portion inside granule indicate space of substrate depletion. (a) and (b) are respectively for higher (25-15 °C) and lower (15-5 °C) range of psychrophilic temperature.

The larger granule size does not always indicate high stability and high settleability of granules. Sometimes, large granule sizes may be connected with 'hollowing'. Feed may penetrate the granule by diffusion only, so when the size of the granule exceeds a certain limit, the concentration of feed in the core of the granules becomes too small to feed the bacteria at this location resulting in the starvation of the microbial population and subsequent autolysis. It is expected that autolysis products are not as densely packed as viable organisms (Bochem et al. 1982) and gas may be entrapped, decreasing the density and settleability of granules. Because of the observed large granule size distribution at all temperatures and HRTs, it can be concluded that during normal ASBR operation, granules will not break or disintegrate by external shear forces or internal gas production.

CONCLUSIONS

Based on this research, the following conclusions are evident:

- The size distribution in the ASBR appears to change with the change of temperature at psychrophilic conditions. From the 25 to 15 °C, the granule size increased with the decrease in temperature and then decreased as the temperature was decreased to 5 °C.
- An equivalent size diameter of about 2-3.3 mm was the predominant size distribution for all temperatures and HRTs. The large size indicated that the loss of large granules with the liquid effluent was minimal.
- The fine percentages are very low at all conditions. This may be due to the loss of the poorly settleable fines with the effluent or from low influent substrate concentration.
- There was no correlation found between temperature and ash content. However, there was a positive correlation between VSS density and ash content. VSS density increased with the ash content.

- The granule size distribution can be explained by the formation of fine particles, and wash out of those fine particles, leaving the larger particles in the reactors.
- The model adequately described the biomass size increase at temperature range 25-15 °C and decrease at 15-5 °C.

CHAPTER 7

LOW TEMPERATURE EFFECTS ON ANAEROBIC MICROBIAL KINETIC PARAMETERS

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ABSTRACT

Anaerobic sequencing batch reactors (ASBRs) were operated in the temperature range of 5 to 25 °C and 24, 16, 12, 8, 6 hour hydraulic retention times (HRTs) over a period of two years to evaluate the effects of different temperatures on the microbial kinetic parameters K , K_s , K_d , Y_g and μ_m . The values of K , K_d and μ_m were found to decrease, and the half saturation constant, K_s , was found to increase with a decrease in temperature in accordance with the Arrhenius equation. Temperature coefficient, θ values for substrate utilization rate, maximum specific growth rate, decay rate and half saturation constant were 1.083, 1.062, 1.076 and 1.05 respectively. Through the determination of kinetic parameters, a design engineer may be able to predict the performance of ASBR treatment systems over a wide range of low temperatures.

Key Words: Anaerobic, Kinetics, Low temperature, Arrhenius equation, Monod equation

INTRODUCTION

The ability to predict effluent quality in a wastewater treatment system requires accurate kinetic models and reliable estimates of kinetic parameters. Sound engineering judgment can best be applied to decisions concerning biochemical processes if it is possible

to quantify the various factors influencing them. Quantification, in turn, depends on the development of system-descriptive mathematical models and their parameters. Efforts during the last three decades in the field of microbial kinetics have led to models of sufficient generality to allow for an adequate description of a diverse number of biochemical processes, including removal of soluble organics in wastewater treatment. These models express specific growth rate as a function of growth limiting substrate concentration, and also of physical characteristics of a reactor system. The performance of a system depends on a group of system specific parameters, values of which depend upon the type of substrate employed, the nature of the bioculture, the pH and the temperature of the medium in conjunction with one or more of process variables.

Several studies have established that significant variations in temperature occur in a wastewater treatment facility and result in variable treatment performance [Brown and Rose, 1969; Grady and Lim, 1980; Lawrence and McCarty, 1970; Pavlosthathis and Giraldo-Gomez, 1991]. Temperature can exert an effect upon a biological system: by influencing the rates of enzymatically driven reactions and by affecting the rate of diffusion of substrate to the biomass [Grady and Lim, 1980]. The design should provide sufficient flexibility for the adjustment of the process in a rational manner so that system-descriptive parameters can be expressed as functions of temperature.

Two biological process models have a wide application in the field of wastewater treatment, relating specific growth rate and substrate concentration. One employs a first-order function so that system equations require three parameters [Eckenfelder and Englands, 1971]. The other uses a hyperbolic function, which deals with four parameters [Lawrence and McCarty, 1970; Topiwala and Sinclair, 1971]. Previous studies have succeeded in quantifying temperature effects in the first-order model through the use of modified van't Hoff- Arrhenius equations [Lawrence and McCarty, 1970; Benedeck and Farkas, 1971]. Although the use of first-order kinetics is valid at low substrate concentrations, system

equations resulting from its use are less general than those obtained from the hyperbolic function [Lawrence and McCarty, 1970; Gaudy and Gaudy, 1966]. Thus there is a need to quantify the effects of temperature on the four parameters in that model as well.

Above all, the kinetics research on biological systems have primarily concentrated on aerobic systems and in the mesophilic temperature range (20-40 °C) in the past [Pavlostathis and Giraldo-Gomez, 1991]. Very few studies have been conducted to determine the system-descriptive parameters for the anaerobic biological systems, operated in the low temperature range (5-25 °C). Anaerobic sequencing batch reactors (ASBR) were used to determine the temperature effects upon a microbial population, assuming that steady-state conditions were achieved. As a continuous stirred tank reactor (CSTR), ASBR works almost continuously at low hydraulic retention times (HRT) due to low cycle time. Sung and Dague developed an ASBR kinetic model for design in the mesophilic temperature range but did not quantify the kinetic parameters [Sung and Dague, 1996].

The purpose of this paper is to illustrate the effects of low temperature on each of the four kinetic parameters (K , K_s , K_d , Y_g) and to quantify those effects.

MODEL INVESTIGATION

The biochemical process consists of three simultaneous events: 1) biomass growth; 2) biomass decay; and 3) substrate utilization. Biomass growth (r_{xg}) with respect to the biomass concentration (X) in the reactor occurs at a first order rate:

$$r_{xg} = \mu(S)X \quad (i)$$

The specific growth rate constant, $\mu(S)$ is a function of the concentration of growth limiting substrate, S . Although many expressions have been proposed to represent $\mu(S)$, the Monod equation [Monod, 1949] was chosen for this work because it has been shown to be satisfactory when a parameter such as biodegradable chemical oxygen demand (COD) is used as a measure of growth limiting substrate

$$\mu(S) = \frac{\mu_m(T) \cdot S}{K_s(T) + S} \quad (\text{ii})$$

Monod kinetics for substrate utilization :

$$U = \frac{K(T) \cdot S}{K_s(T) + S} \quad (\text{iii})$$

Parameters $\mu_m(T)$, $K(T)$ and $K_s(T)$ are assumed to be functions of temperature. Biomass decay is assumed to occur in a first-order manner with the rate constant K_d , as a function of temperature:

$$r_{xd} = K_d \cdot X \quad (\text{iv})$$

Finally, substrate utilization rate occurs at a rate which is proportional to the specific growth rate of the biomass:

$$r_s = \frac{\mu(S) \cdot X}{Y_g(T)} \quad (\text{v})$$

The term $Y_g(T)$, represents the true growth yield, i.e., the quantity of biomass formed per unit of substrate removed. $Y_g(T)$ can not be measured directly because a portion of biomass is always lost due to decay.

By the use of the presented fundamental kinetic relationships and applying mass balances for the biomass and substrate, explicit equations have been developed assuming ASBR can achieve steady-state conditions. The steady state reactor biomass concentration (X) and effluent substrate concentration (S_e) are given by the following equations [Metcalf & Eddy, 1991]:

$$X = \frac{Y_g \cdot (S_o - S_e)}{(1 + K_d \cdot \text{HRT})} \quad (\text{vi})$$

and

$$S_e = \frac{K_s(1 + \text{HRT} \cdot K_d)}{(\text{HRT} \cdot (Y_g \cdot (K - K_d) - 1))} \quad (\text{vii})$$

where HRT = hydraulic retention time. S_o = influent substrate concentration (mg l^{-1})

Equations (vi) and (vii) describe the performance of ASBR at various hydraulic retention times and temperatures, assuming influent waste is soluble and no biomass exists in the influent. The experimental method uses HRT as an independent variable while measuring X and S_e at a constant temperature. The data may then be used to determine the four parameters, $K(T)$, $K_s(T)$, $K_d(T)$, and $Y_g(T)$ and subsequently μ_m . This procedure is repeated several times at different temperatures so that the effect of temperature upon the parameters may be evaluated.

MATERIALS AND METHODS

Reactors

Three identical ASBRs of 6 L effective volume were used in this study. The reactors were cylindrical in shape with an internal diameter of 113 mm and were made using 12 mm thick Plexiglas. The ASBR experimental setup is shown in Figure 7-1. Mixing was provided by a gas recirculation system working every 30 minutes for 30 seconds.

Substrate

As reported in an earlier research on the ASBR at Iowa State, the substrate used in this study was a synthetic waste which consisted of non-fat dry milk (NFDM), sodium bicarbonate, and a trace mineral solution. The characteristics of the NFDM are available elsewhere [Banik and Dague, 1996a]. Sodium bicarbonate (NaHCO_3) was used to maintain suitable buffering. Five trace minerals (Fe, Zn, Ni, Co, Mo) were added to the substrate to provide adequate nutrients for the granules [Banik and Dague, 1996a].

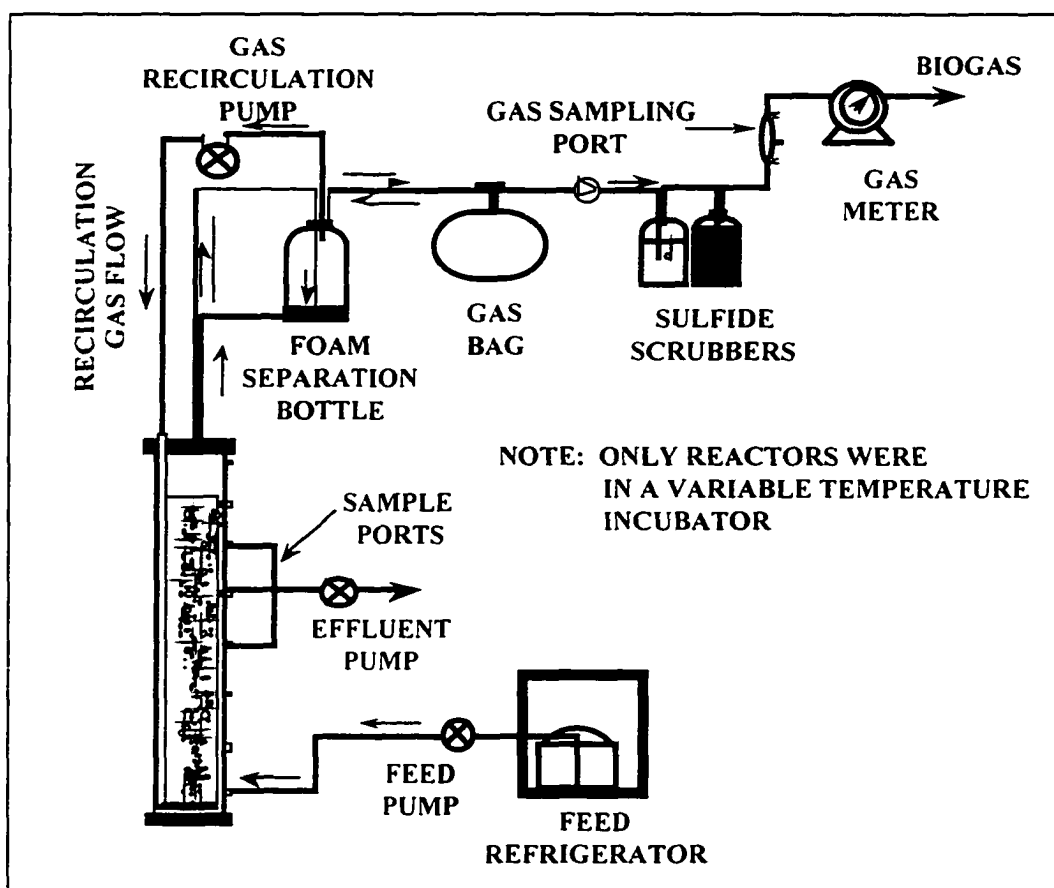


Fig. 7-1. ASBR setup for the dilute wastewater treatment.

The reactors were fed with the NFDM substrate with a COD concentration of 600 mg l^{-1} and a BOD_5 concentration of 285 mg l^{-1} , typical of a dilute wastewater such as municipal wastewater. The influent COD concentration was kept constant so that temperature and HRT variation effects could be determined independently. Feed was prepared daily and supplied to the reactors from a refrigerator.

Biomass Seed

All three reactors were inoculated with elutriated mesophilic granular methanogenic sludge obtained from an ongoing ASBR pilot-plant, operated at 35°C at the Water Pollution Control Plant at the City of Cedar Rapids, Iowa. Sufficient granular sludge was added at

start-up to bring the MLSS to approximately 12 gL⁻¹. The reactors were operated inside the incubator, maintaining the desired temperature. Consequently, the actual temperature of the reactor contents were as much as ± 0.5 °C from the desired value.

Experimental Design

Five hydraulic retention times (HRT) 24, 16, 12, 8 and 6 hours were employed for a given temperature to determine the kinetic parameters. Furthermore, eight temperatures 25, 20, 17.5, 15, 12.5, 10, 7.5 and 5 °C were investigated.

Start-up and Operation

Reactor operation started with a substrate COD concentration of 1000 mgL⁻¹ at 25 °C at an HRT of 24 hours. Following the achievement of steady-state conditions, the substrate COD concentration was changed from 1000 mgL⁻¹ to 600 mgL⁻¹. Each reactor was operated at two HRTs at each temperature. The operating temperature was lowered first from 25 °C to 20 °C and then lowered from 20 °C to 5 °C in steps of 2.5 °C. First, the reactors were operated at a specific temperature with HRTs of 24, 16, and 12 hours. After collecting steady-state data at each temperature, HRTs were changed to their subsequent respective lower values of 12, 8 and 6 hours. Between each subsequent data point, the reactors were operated for a considerable time at the same HRT and temperature to ensure that the data reflected true steady-state conditions. Subsequent temperature changes were accomplished by lowering the temperature by 0.5 °C in every two days. This gradual lowering of the temperature was used to ensure that granules would not be washed out of the system while the microorganisms were acclimating to the lower temperature. The HRTs were also changed slowly to protect against excessive biomass washout.

Method of Analyses

To determine the performance and stability of the reactors, pH determinations and gas measurements were made every day. Alkalinity, VFA, COD, solids and gas characteristics were measured once per week. In each steady-state condition, the parameters were determined three times and the results averaged to obtain a data point. Analyses for BOD₅, sulfate, sulfide and automated image analysis were also done for each data point.

Methanogenic activity tests were also conducted several times.

Alkalinity, VFA, COD, BOD₅, sulfate, sulfide and solid level were determined according to Standard Methods [1995].

Method of Calculation

The kinetic parameters [$K(T)$, $K_s(T)$, $Y_g(T)$, $K_d(T)$] were determined from linearized forms of equations (vi) and (vii). K and K_s values were calculated for each temperature by fitting the data to the equation

$$\frac{X.HRT}{(S_o - S_e)} = \frac{K_s}{K \cdot S_e} + \frac{1}{K} \quad (viii)$$

by least-squares analysis using the mean values of S_e and X associated with each value of HRT.

Similarly, $Y_g(T)$ and $K_d(T)$ were determined by fitting the linearized form of equation

$$\frac{1}{SRT} = \frac{Y_g(S_o - S_e)}{X.HRT} - K_d \quad (ix)$$

Using the determined values of $K(T)$ and Y_g , maximum specific growth rate, μ_m can be calculated from the equation:

$$\mu_m = K \cdot Y_g \quad (x)$$

RESULTS AND DISCUSSIONS

Table 7-1 presents a summary of results from three ASBRs.

Because of the nature of the microbial population employed, it was expected that changes in culture might influence the results [Chiu, 1972]. Consequently, quantitative observations of culture color and morphology were made throughout the experiments. No change of color was apparent, however, changes were noted in cultural morphology. The size of the biomass increased with a decrease of temperature from 25 to 15 °C and biomass size decreased from 15 to 5 °C. But it was not possible to detect any changes in the data which could be attributed to these morphological differences. Layering was observed at 5 °C, which confirm temperature may have a role on layering [Banik et al. 1997a].

Within the field of microbial kinetics the expression most commonly used to quantify the effects of temperature on reaction rates is the Arrhenius equation [Levenspiel, 1972]. Although originally proposed to describe the effects of temperature on the rates of chemical reactions, the Arrhenius equation can be extended to study the influence of temperature on the rates of biological process [Grady and Lim, 1980] as:

$$K = Ae^{-E/RT} \quad (xi)$$

in which K = the reaction rate constant or maximum substrate utilization rate (d^{-1});

T = the absolute temperature in Kelvin;

R = the ideal gas constant ($cal.mole^{-1}.^{\circ}K^{-1}$);

A = frequency factor;

E = activation energy ($cal.mole^{-1}$).

The ASBRs were started-up at 25 °C with a mesophilic granular biomass and the reactor temperatures were lowered to 5 °C over time. Since psychrophilic bacteria do not

TABLE 7-1. Summary of results of three ASBR at different temperature and HRT

Temp. (°C)	Reciprocal Temp. (1000(°K ⁻¹))	HRT (hours)	Effluent SCOD (mg/l ⁻¹)	SCOD Removal Rate (%)	Reactor MLVSS (mg/l ⁻¹)
25	3.354	24	9	98.5	8980
		16	15	97.5	9860
		12	23	96.3	8890
		8	26	95.7	9750
		6	30	95	9330
20	3.411	24	16	94.2	8340
		16	28	95.3	9410
		12	35	94.2	9660
		8	45	92.5	9260
		6	52	91.3	9120
17.5	3.44	24	31	89.7	7540
		16	54	91	8620
		12	52	91.2	7960
		8	68	88.7	8450
		6	98	83.7	8930
15	3.47	24	40	86.3	7330
		16	60	90	8410
		12	80	85.3	7950
		8	91	84.8	8350
		6	111	81.5	8690
12.5	3.5	24	48	83	6980
		16	88	85.3	8670
		12	81	86.5	7870
		8	98	83.7	8320
		6	132	78	8870
10	3.532	24	78	77.2	6440
		16	135	77.5	8160
		12	134	77.7	7780
		8	158	73.7	8340
		6	189	68.5	8640
7.5	3.563	24	90	72	6470
		16	148	75.3	7890
		12	148	75.2	7850
		8	188	68.7	8120
		6	212	64.7	9210
5	3.595	24	140	67.7	6020
		16	172	71.3	7650
		12	183	72.8	7080
		8	198	67	7520
		6	228	62	8230

grow well or may not grow at all under mesophilic temperatures, it is most likely that many of the organisms in the mixed cultures would be psychrotrophic rather than psychrophilic. In the above circumstances, it was decided not to split the analyses between the psychrophilic range (5 to 15 °C) and the mesophilic range (20 to 25 °C), although such analyses may be supported by the broken Arrhenius plots in some cases.

Of the four parameters investigated in this study, $K(T)$ and $K_d(T)$ are reaction rate constants and would be expected to be influenced by temperature in a manner similar to equation (xi). Table 7-2 shows that $K(T)$ and $K_d(T)$ values decreased with a decrease in temperature. Figure 7-2 shows a plot of $K(T)$ as a function of absolute temperature in the manner of equation (xi). The activation energy E_a was found to be 126770 cal.mole⁻¹, which is similar to the values obtained by other investigators [Grant and Lim, 1980; Viraraghavan, 1996]. The negative $K(T)$ value at 5 °C showed that ASBR at this temperature did not follow the Monod and the Arrhenius equations although experimental soluble COD and BOD₅ removals were in the range of 62-73% and 75-90% respectively [Banik and Dague, 1996a].

Table 7-2 Kinetic Parameters

Temp., (°C)	$K(T)$, (d ⁻¹)	$K_s(T)$, mg/l	K_d , (d ⁻¹)	$Y_g(T)$	$\mu_m(T)$, (d ⁻¹)
25	1.59	218.6	.0062	.149	.237
20	1.14	259.5	.0043	.129	.147
17.5	1.12	448.8	.005	.138	.155
15	1.06	534.3	.0041	.145	.154
12.5	0.69	395.2	.0029	.153	.106
10	0.53	476.6	.0027	.178	.094
7.5	0.42	424.0	.0017	.183	.077
5	-0.42	811.2	.0014	.165	-.069

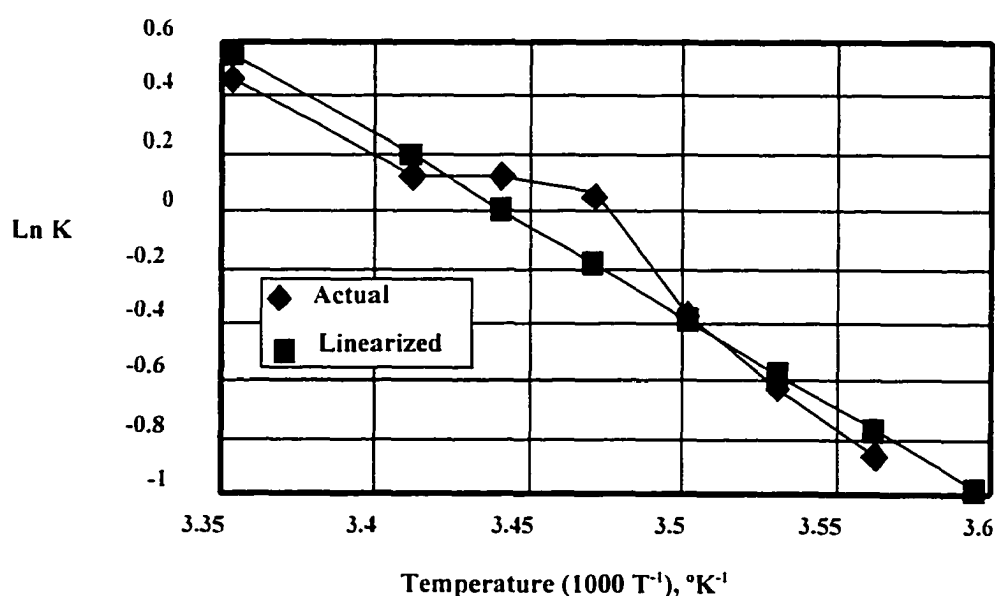


Fig. 7-2. Variation of K values at different low temperatures ($\ln K = 21.95 - 6380(T^{-1})$; $R^2 = 0.93$).

The value of temperature coefficient θ was found to be 1.083 where $\theta_{25-7.5\text{ C}} = e^{6380/(298.15 \cdot 280.65)}$.

Table 7-2 shows that the values of K_d are in the range of 0.0062-0.0014 d⁻¹ and decreased with a temperature decrease. Sherrard [1971] found that the K_d values were 0.0018 h⁻¹, treating sucrose and peptone, and 0.0033 h⁻¹ treating peptone in a mixed culture. Figure 7-3 shows the values of specific decay rate constant, K_d , plotted as a function of temperature. From the plot, the activation energy was determined to be 12140 cal.mol⁻¹, which can also be modeled from the Arrhenius equation. The θ values for decay constant was found to be 1.076. Benedeck and Farkas [1971] reported that the rate of endogenous oxidation in batch experiments followed equation (xi) with an activation energy of 13150 cal.mole⁻¹. Furthermore, in studies using pure and mixed cultures (Table 7-3), similar values of activation energy were obtained at different temperatures. The ratio of the two values, θ_{K_d} / θ_K , was 0.993 and E_{K_d} / E_K , was 0.96. For an aerobic system, the E_{kd} / E_K values are

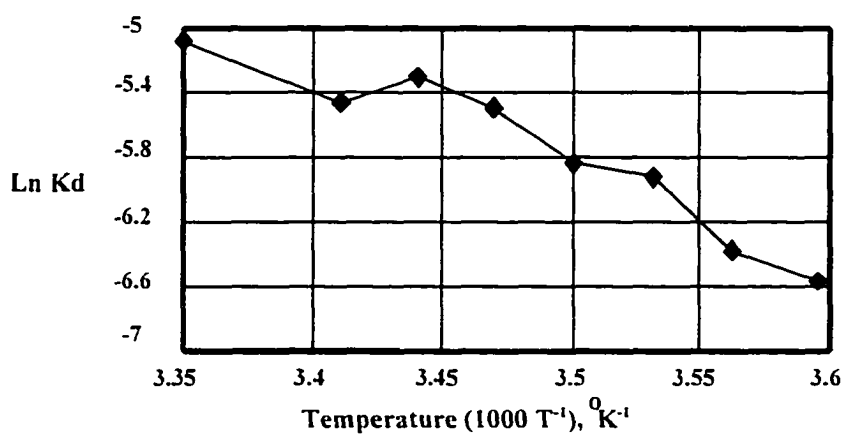


Fig. 7-3. Variation of decay rate constant at various low temperatures ($\ln K_d = 15.53 - 6110 (T^{-1})$; $R^2 = 0.92$).

Table 7-3 Variation of Arrhenius activation energies for bacterial decay rate constant

Temp. °C	No. of points	Organisms	Growth Medium	E. Calories/mol	Source
20-30	3	<i>P.fluorescens</i>	Glucose	18200	[Mannet]
8-30	3	<i>P.fluorescens</i>	Glucose	8430	[Palumbo]
25-40	4	<i>A.aerogenes</i>	Glucose	9000	[Topiwala]
10-40	4	Mixed cultures	Glucose	13900	[Muck]

close to 1.10 [Mannet and Nakayama, 1971]. θK_d and θK values suggest that as a general rule the specific decay rate constant is influenced by temperature in a manner similar to the maximum substrate utilization rate.

The fact that K_d decreased as the temperature was decreased did not necessarily imply a reduction in the net amount of biomass formed during growth. This will depend upon the effects on the yield as well. Topiwala and Sinclair [1971] and Tempest and Hunter [1965] observed the fact that much of the variation in observed yield was caused by the change in decay rate. Figure 7-4 presents the effects on the true growth yield observed in this work along with values obtained by others [Brown and Rose, 1969; Topiwala and Sinclair, 1971; Palumbo and Witter, 1969; Muck and Grady, 1974 and this work]. From the examination of the data, it can be concluded that temperature effects on yield was negligible.

Benefield and Randall [1975] found that within the temperature range of 11-35 °C and under batch conditions, the specific growth rate, and specific substrate utilization rate, U vary at the same rate when the temperature of the system is changed. Considering that $\mu = Y_{obs} \cdot U$, which suggests that if the treatment system could be adjusted through operation to compensate for the variation in metabolic activity that occurs with the temperature change, steady state conditions observed by Y_{obs} and effluent quality can be maintained. As the temperature decreased, S_e increased as well as U decreased. Since μ remained constant and U decreased, the observed yield values for such a system will increase with a decrease in temperature. Such a phenomenon was observed by Topiwala and Sinclair [1971]. The writer also observed the same trend in the temperature range 20 -7.5 °C. The mean value of yield was found to be 0.15 mg /mg of COD utilized with a standard deviation of 0.018. Pavlosthtathis and Giraldo-Gomez [1991] reported that anaerobic yield values for carbohydrate and protein were 0.35 and 0.20 respectively. In an anaerobic system, biomass synthesis is not only directly related to the amount of COD removed as in an aerobic system,

but is also related to the class of organics being treated [Speece, 1996]. Microbes convert more complex organics primarily to acetate and H_2 , which are subsequently converted to methane by the methanogens. The protein class of organics provides relatively less energy to these organisms than carbohydrates. Since high percentages of protein (>36%) exist in the influent [Banik and Dague, 1996a], experimental data showed in a low synthesis.

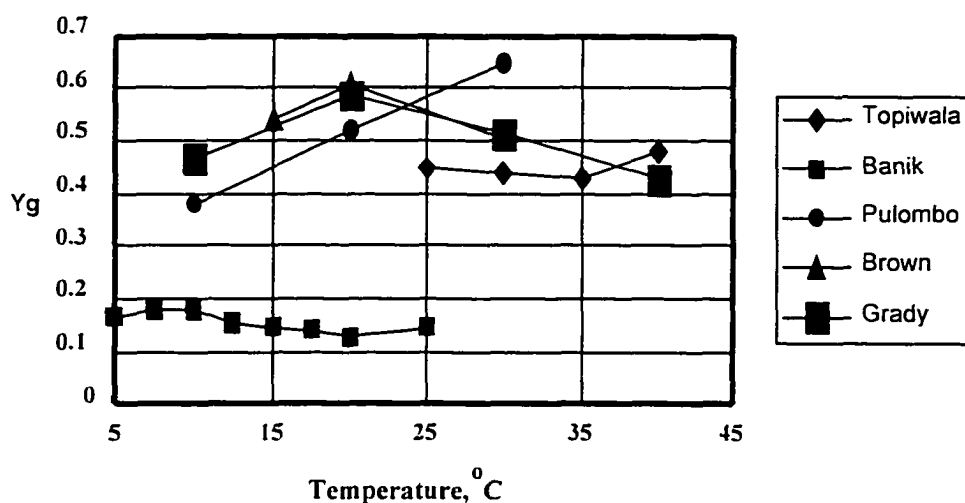


Fig. 7-4. Variation of yield at various temperatures

The last parameter considered in this study was the saturation constant, $K_s(T)$. Unlike $K(T)$ and $K_d(T)$, the saturation constant is not really a rate constant [Muck and Grady, 1974], but is rather a factor which quantifies the effect of substrate concentration on the growth rate. Topiwala and Sinclair [1971] have stated that $K_s(T)$ could be considered to be related to the basic substrate transport process into the cells. As a consequence, they found that the reciprocal of $K_s(T)$ could be plotted satisfactorily on an Arrhenius plot. In their plot, $K_s(T)$ decreased with an increasing temperature. Lawrence and McCarty [1970] also reported similar results, treating acetate with methanogenic bacteria. In their study, K_s for acetate conversion to methane increased from 164 to 2130 mg/l as the temperature decreased from 35 to 20 °C but K values decreased from 6.67 to 3.85 day^{-1} . Viraraghavan and Varadarajan [1996] mentioned that K_s values increased as the temperature increased for anaerobic filter

treatment of septic tank effluent from 5 to 20 °C and dairy wastewater from 12.5 to 30 °C but decreased for whey wastewater from 16 to 30 °C. The results of their study, however, did not indicate any clear effects of temperature upon $K_s(T)$. Other researchers have reported an increase in $K_s(T)$ with an increase in temperature [Jones and Hough, 1946; Knowles et al. 1965; Muck and Grady, 1974]. Grant and Lin also found $K_s(T)$ increased with an increase in temperature, treating synthetic substrate with UASB [1995]. Consequently, at this time, it appears that saturation constant may either increase or decrease with decreasing temperature, depending upon the organisms, environmental conditions and reactor configurations. However, the data presented here indicate a general increase in $K_s(T)$ with a decrease in temperature as observed by Lawrence and McCarty [1970] and Topiwala and Sinclair [1971] (Figure 7-5). The activation energy E and θ values from this figure were found to be 8420 cal.mol⁻¹ and 1.05 respectively.

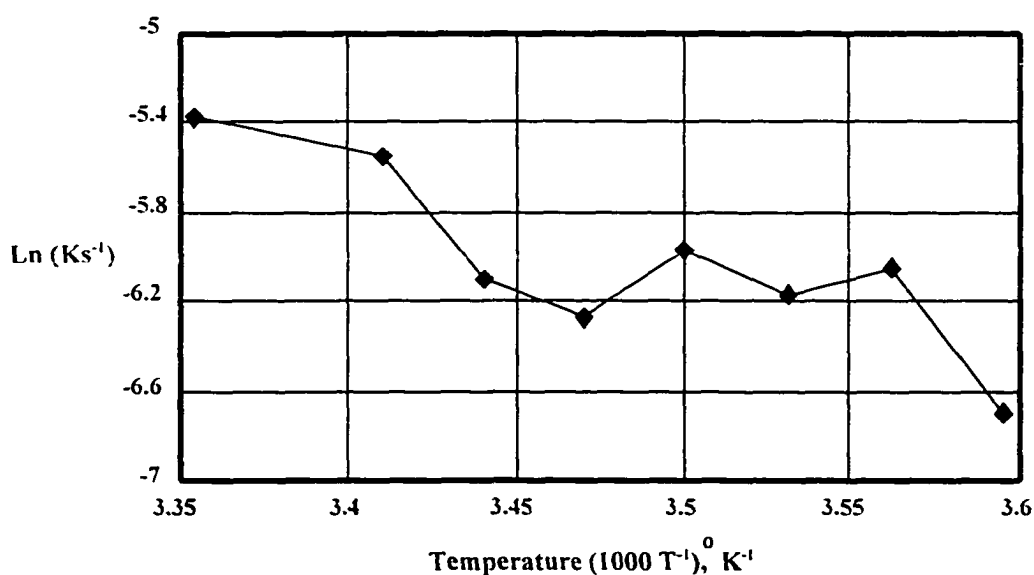


Fig. 7-5. Variation of K_s values at various low temperatures ($\ln(K_s^{-1}) = 8.72 - 4240(T^{-1})$; $R^2 = 0.70$)

Many researchers [Greene and Jezeski, 1954; Kato et al., 1994; Muck and Grady, 1974] associated $K_s(T)$ with diffusion and other transport mechanisms. Consequently, the manner in which it varies with temperature, will depend upon the physical environment in which the cultures are grown.

The plot of μ_m vs. $(1/T)$ in Figure 7-6 presents the effect of temperature on maximum specific growth rate. From Figure 7-6, activation energy was determined to be 10500 cal.mole⁻¹. Activation energies which appear typical for different temperatures and organisms are shown in Table 7-4. θ values for this plot was found to be 1.06. The value of

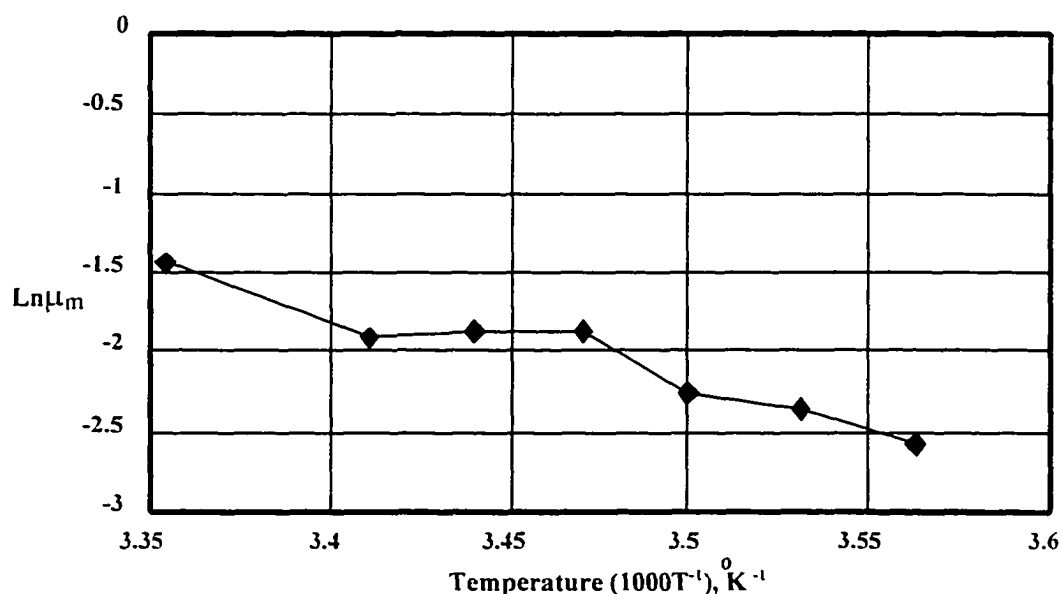


Fig. 7-6. Variation of maximum specific growth rate at various low temperatures ($\ln \mu_m = 15.5 - 5060 (T^{-1})$; $R^2 = 0.923$.

$K_d/\theta \mu_m$ is 1.015. A similar comparison of $E K_d/E \mu_m$ from the Marnett and Nakayama [1971] study showed a value of 1.10, while a value of 1.07 was observed from Topiwala and Sinclair [1971]. The similarity of these values shows that the specific decay rate constant is influenced by temperature in a manner similar to the maximum specific growth rate constant. The summary of activation energy and θ values for maximum substrate utilization rate, maximum specific growth rate, decay rate and half velocity constant is shown in Table 7-5.

Table 7-4. Typical values of the Arrhenius Activation Energies (E) for the maximum specific growth rate

Temp. °C	Organisms	Growth Media	E. cal.mole ⁻¹	Source
25-40	<i>A. aerogens</i>	Glucose	8400	[Topiwala]
20-28	<i>Gibberella fujikuroi</i>	Glucose	5100	[Borrow]
20-30	a psychrophil	Glucose	10000	[Brown]
10-30	Mixed culture	Glucose	12500	[Muck]
0-30	<i>Pseudomonas</i> sp.	Lactose	20500	[Greene]

θ

Table 7-5 Summary of activation energy and θ values for different kinetic parameters.

	Maxm. substrate utilization rate, K (d ⁻¹)	Maxm. sp. growth rate μ_m (d ⁻¹)	Decay rate, K _d (d ⁻¹)	Half velocity constant (K _s) (mg l ⁻¹)
E.cal.mole ⁻¹	12670	10500	12140	8420
θ	1.083	1.062	1.076	1.05
R ²	0.93	0.92	0.93	0.70

The values of μ_m and K_s depend on substrate employed and the organism present in the system besides temperature. Generally substrates which are difficult to degrade will be characterized by low values of μ_m and high values of K_s [Speece, 1996]. This is true for the experimental data, with high percentages of protein in substrate. The values of μ_m are very low in comparison to aerobic systems but the K_s values were high in comparison to the existing literature [Grant and Lim, 1980].

The analysis so far has centered around the effects of temperature upon the four individual parameters of microbial kinetics K(T), K_s(T), Y_g(T) and K_d(T), and μ_m . Of these K(T), K_s(T), K_d(T) and μ_m were shown to be affected by temperature in a manner which

can be described by an Arrhenius equation. The effects upon $K(T)$ and $Y_g(T)$ were less clear-cut, although both were shown to be influenced by temperature. It should be recognized, however, that the effect of temperature upon the performance of a biological reactor depends upon the interaction of all kinetic parameters.

The system-descriptive parameters analyzed in this paper are independent of the operational conditions imposed upon the wastewater treatment system. Equations similar to 6 and 7 can be written for other biological systems [Lawrence and McCarty, 1970]. Through the use of kinetic parameters, design engineers may be able to predict the performance of a treatment system over a wide range of temperatures and loading conditions [Banik et al., 1997b].

SUMMARY AND CONCLUSIONS

Laboratory studies of ASBR using a synthetic substrate were performed to assess the effects of low temperature upon each of the four parameters required to characterize microbial kinetics. As was anticipated from the results of others, pure and mixed microbial culture, the maximum substrate utilization rate, maximum microbial growth rate and the bacterial decay rate constant were found to decrease, and half saturation constant was found to increase with a decrease in temperature in accordance with the Arrhenius equation over the temperature range of 25-7.5 °C. The θ values for substrate utilization rate, maximum specific growth rate, decay rate and half saturation constant were 1.083, 1.062, 1.076 and 1.05 respectively. The true growth yield was found to change negligibly with a decrease in temperature, and was 0.15. The yield value was low due to a high percentage of protein present in the substrate. It appears that in a physical system, substrate type and microbial composition may have influence on the effects of temperature upon the saturation constant. At 5 °C, the $K(T)$ and μ_m values were negative, because ASBR kinetics did not follow the Monod and the Arrhenius equations at that temperature.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

The research presented in this document resulted in several significant findings. Further discussion of each of these points may be found in the previous Results and Discussions sections. After careful analysis and review of the data from these experiments, the following conclusion and recommendations can be drawn:

1) The ASBR process has intrinsic characteristics that result in a high level of organic removals, even when treating a low strength wastewater at temperatures from 25 °C to 5 °C. At 20 to 25 °C, the soluble COD and BOD₅ removals were in excess of 90%. Even at a low temperature of 5 °C and a six hour HRT, BOD₅ removal was 75%.

2) The VFA concentration was very low (8 - 40 mg/L) at various temperature and loading concentrations, without accumulation with time. This demonstrates that the ASBR can be effectively used at very low temperatures to remove organics at organic loading 2.4 gCOD/L/day.

3) The effluent suspended solids concentrations tended to increase with increases in organic loading and/or decreases in temperature, but the effluent solids losses were fairly compensated for by increases in biomass growth and retention. SRT at 5 °C and at HRT 6 hr was about 25 days, which was high enough to maintain very good process performance. This also demonstrated that low F/M during decanting play a significant role for selection and retention of biomass.

4) Psychrophilic temperatures did not have a dramatic effect on the microstructure of the ASBR granules, with the possible exception of encouraging a layered structure at the lower (5 °C) temperature. Granules grown on non-fat-dry-milk at 15 and 25 °C exhibited a rather uniform structure, demonstrating the absence of a layered structure. This confirms the

hypothesis that granule microstructure depends to a large extent on the nature of the substrate.

5) The granules at all temperature consisted of predominantly *Methanothrix*-like microorganisms as the key structural element, which seems to suggest that *Methanothrix* plays an important role in granulation and retention in high-rate anaerobic processes such as the ASBR .

6) The specific methanogenic activity (SMA) of granules grown at psychrophilic temperatures were slightly less than that of granules grown at mesophilic temperatures. The production of methane at 25 - 5 °C from granules grown at mesophilic temperatures indicated the existence of psychrophilic microorganisms that could grow at both mesophilic and psychrophilic temperatures.

7) The specific methanogenic activity (SMA) for granules biodegrading mixtures of acetate and propionate was significantly lower than that for acetate alone. This established that biological treatment performance is substrate dependent, and decrease with the complexity of oorganics.

8) The size distribution in the ASBR appears to change with the change of temperature at psychrophilic conditions. From the 25 to 15 °C, the granule size increased with the decrease in temperature and then decreased as the temperature was decreased to 5 °C. An equivalent size diameter of about 2-3.3 mm was the predominant size distribution for all temperatures and HRTs. The large size indicated that the loss of large granules with the liquid effluent was minimal due to their high settleability.

9) The fine percentages are very low at all conditions. This may be due to the loss of the poorly settleable fines with the effluent or from low influent substrate concentration.

10) There was no correlation found between temperature and ash content. However, there was a positive correlation between VSS density and ash content. VSS density increased

with the ash content. The granule size distribution can be explained by the formation of fine particles, and wash out of those fine particles, leaving the larger particles in the reactors.

11) The maximum substrate utilization rate (K), maximum microbial growth rate (μ_m) and the bacterial decay rate constant (K_d) were found to decrease, and half saturation constant (K_s) was found to increase with a decrease in temperature in accordance with the Arrhenius equation over the temperature range of 25-7.5 °C.

12) The temperature coefficient (θ) values for substrate utilization rate, maximum specific growth rate, decay rate and half saturation constant were 1.0833, 1.062, 1.076 and 1.05 respectively, very consistent with the existing literature.

13) The true growth yield was found to change negligibly with a decrease in temperature, and was about 0.15 mg of biomass/mg of COD utilised. The yield value was low due to a high percentage of protein present in the substrate. Another cause may be less energy available due to the conversion of methane.

14) It appears that in a physical system, substrate type and microbial composition may have influence the effects of temperature upon the saturation constant.

15) At 5 °C, the K (T) and μ_m values were negative, because ASBR kinetics did not follow Monod and Arrhenius equations at that temperature, or may be due to the change of composition of microorganism.

The results revealed that the application of the ASBR to the treatment of low strength, low temperature wastewaters offers the possibility of lower cost treatment of industrial and municipal wastes that are normally treated aerobically with higher expenditures of energy and increased sludge production.

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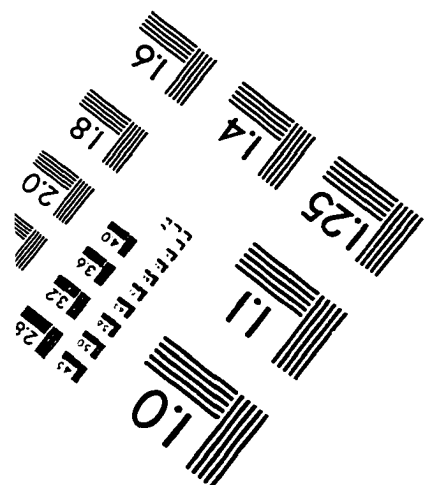
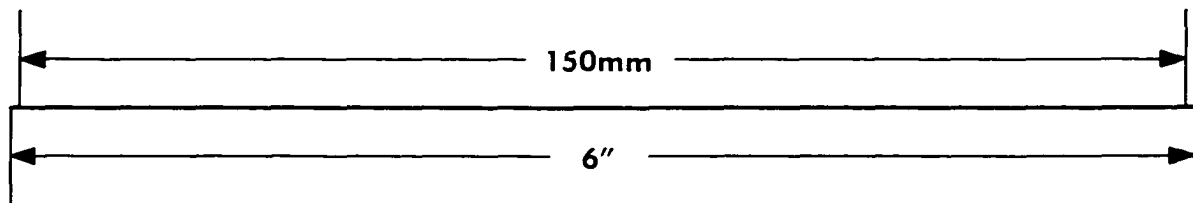
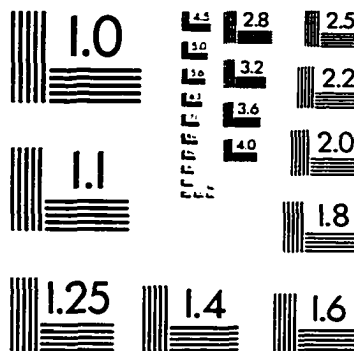
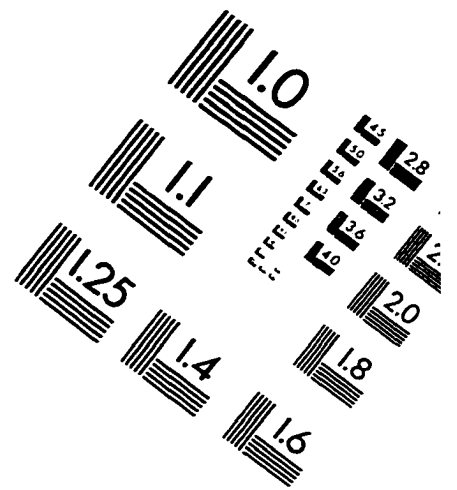
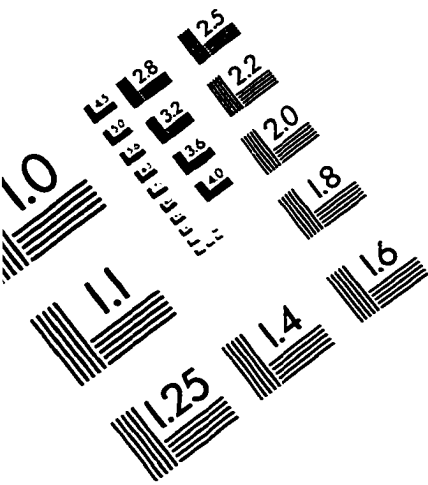
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IMAGE EVALUATION TEST TARGET (QA-3)



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